5th Mechanobiology Conference
Organized by the Mechanobiology Institute, Singapore (MBI)

The Mechanobiology of Multicellular Systems

Program & Abstracts

9 - 11 November 2011
Shaw Foundation Alumni House, NUS
The 5th Mechanobiology Conference

The Mechanobiology of Multicellular Systems

9 - 11 November 2011
Shaw Foundation Alumni House
National University of Singapore

Organized by the Mechanobiology Institute, Singapore (MBI)
The 5th Mechanobiology Conference:
The Mechanobiology of Multicellular Systems

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Yusuke Toyama, training workshop co-chair
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Evelyn Yim, abstract book
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Siti Haryanti, training workshop

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Welcome to Conference Participants

Welcome to the 5th Mechanobiology Conference organized by the Mechanobiology Institute, Singapore.

The annual Singapore Mechanobiology meetings provide a wonderful opportunity to watch and promote the exciting progress in this exponentially developing field. It is becoming increasingly clear that the mechanobiological perspective is indispensable to the understanding of basic living phenomena – from the dynamics of individual biological macromolecules, through molecular processes of self-assembly and force generation, to cell division, migration, adhesion, mechanotransduction and finally cell differentiation and the development of tissue architecture.

This year, we focus on “The Mechanobiology of Multicellular Systems” as a major subject of the conference. Formation, self-organisation and functioning of multicellular systems play central roles in embryonic development, inflammation, wound healing, bacterial pathogenesis, and cancer metastasis. This conference will cover recent progress in the basic aspects of cell-matrix and cell-cell adhesion, crosstalk between adhesion and the cytoskeleton and mechanisms of cell mechanosensitivity. Interplay between signal transduction and biomechanical aspects of cell adhesion and cytoskeletal dynamics will be highlighted. We will hear discussions on the organization of collective cell movement and mechanisms of morphogenesis in development. Studies of diverse types of cell collectives and intercellular interactions, from mammalian tissues to bacterial biofilms, will be presented.

Following this Conference, we will also be organising the inaugural satellite training workshop “Forces and Cells” for junior scientists and graduate students (chaired by Profs Lim Chwee Teck and Yusuke Toyama). It will see 30 participants from Singapore, USA, China, Korea, Germany, Australia, India and Japan.

We thank all our speakers and the 340 scientists from 15 different countries for participating in this unique meeting. We also thank all the instructors who will be teaching at our satellite workshop. In addition, we would like to take this opportunity to thank all the sponsors who helped make this event possible.

We appreciate the efforts of the Local Organizing Committee and, in particular, the teams of Conference Secretary Ms. Cynthia Lee and Workshop Secretary Ms. Siti Haryanti who have managed all logistical conference issues in a wonderfully efficient and tireless way.

We look forward to a successful 5th Mechanobiology Conference that will strengthen and deepen our scientific and personal ties.

Alexander D. Bershadsky and G.V. Shivashankar
Conference Chairs
Scientific Program
**Tuesday, 8 November 2011**

Arrival of delegates

04.00 - 06.00 PM  Set up of posters

**Wednesday, 9 November 2011**

07.45 AM  Registration

08.30 AM  Opening Address and Welcome
Alexander BERSHADSKY (Conference Co-Chair)

SESSION 1:
**Cell Mechanosensitivity and Integrin Adhesion**
Choy Leong Hew, Session Chair

08.45 AM  
**Keynote Lecture 1:** Michael SHEETZ
*Cellular Mechanosensing at Multiple Levels*

09.30 AM  
**Benny GEIGER**
Mechanoregulation of Integrin Adhesion Dynamics

10.00 AM  
**Martin SCHWARTZ**
Forces Across Cell Adhesion Receptors

10.30 – 11.00 AM  Tea break

11.00 AM  
**Alexander BERSHADSKY**
Cell-Matrix Adhesion-Dependent Assembly of the Actin Cytoskeleton

11.30 AM  
**Christopher CHEN**
A Materials Approach to Studying Mechanotransduction

12.00 PM  
**Benoît LADOUX**
A Large Scale Mechanosensing Mechanism to Explain the Cell Response to the Stiffness of the Substrate

12.30 PM  
**Martin HUMPHRIES**
Insights From Proteomic Analyses of Adhesion Signalling

01.00 – 2.00 PM  Lunch
### SESSION II:
**Host-Microbe Interactions and Bacterial Collective Behaviour**
Yan Jie, Session Chair

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Thursday, 10 November 2011

**Keynote Lecture 3**

08.45 AM  Timothy SPRINGER  
Biology and Physics of Von Willebrand Factor Concatamers

**SESSION III:**  
**Mechanisms of Cadherin Adhesion**  
Cheng Gee Koh, Session Chair

09.30 AM  Larry SHAPIRO  
Structural Biology of Cadherin-Mediated Cell Adhesion

10.00 AM  Barry HONIG  
Physical Principles of Cadherin-Mediated Cell-Cell Adhesion

10.30 AM  Sergey TROYANOVSKY  
Diversity of Intercadherin Interactions in Adherens Junctions

11.00 – 11.30 AM  Tea break

11.30 AM  Jean-Paul THIERY  
Control of Adhesive Strength in Cadherin-Mediated Adhesion

12.00 PM  Masatoshi TAKEICHI  
Polarized Contractility of Adherens Junctions Leads to Neural Tube Closure

12.30 – 2 PM  Lunch and poster session

**Keynote Lecture 4**

02.00 PM  Keynote Lecture 4 : Alan HALL  
Rho GTPases and the Morphogenesis and Migration of Epithelial Cells
SESSON IV:  
CYTOSKELETON, SIGNALING AND CELL-CELL JUNCTIONS  
Paul Matsudaira, Session Chair

02.45 PM  Alpha YAP  
Translating Time into Space: Regulating the Rho GTPase Cycle at the Epithelial Zonula Adherens

03.15 PM  Vania BRAGA  
Rho GTPases in the Regulation of Epithelial Contraction and Differentiation

03.45 PM  Kozo KAIBUCHI  
Tiam1 Acts with the Par Complex to Control Talin-Mediated Rac1 Activation

04.15 – 4.45 PM  Tea break

04.45 PM  René-Marc MÈGE  
Contribution of Cadherin Anchoring to Actin and Force Transduction In Cell-Cell Contact Maturation and Cell Migration

05.15 PM  Roland WEDLICH-SOLDNER  
Actin Worms and Myosin Networks- Towards an Integrated View of the Actin Cortex

05.45 PM  Yoshimi TAKAI  
Roles of Nectins in Heterotypic Cell Adhesion

06.15 PM  Virginia CORNISH  
TMP-Tag: A Chemical Surrogate to the Fluorescent Proteins for Live Cell Imaging

06.45 PM  End of day 2

07.00 PM  Cocktail reception  
T-building Level 10
SESSION V:
CELL FORCE RESPONSE AND COLLECTIVE CELLMovEMENTS
Boon Chuan Low, Session Chair

08.45 AM  Jeff FREDBERG
Collective Cellular Guidance Guided by Cooperative Intercellular Forces

09.15 AM  Pascal SILBERSAN
Collective Migration of Epithelial Cells: The Role of Leader Cells

9.45 AM  Chwee Teck LIM
Mapping the Collective Migration of Epithelial Cells

10.15-10.45 AM  Tea break

10.45 AM  Roger KAMM
Multi-Cell Interactions in Metastatic Cancer

11.15 PM  Erez RAZ
Motility and Directed Migration of Zebrafish Primordial Germ Cells

11.45 – 2 PM  Lunch and poster session
SESSION VI:
Mechanobiology of Development and Tissue Morphogenesis
Yusuke Toyama, Session Chair

02.00 PM  Thomas LECUIT
The Subcellular Mechanics of Tissue Morphogenesis

02.30 PM  Maithreyi NARASIMHA
The Influence of Mechanics and Chemistry on Spatially
Patterning Cell Behaviour and Tissue Dynamics: Insights from
Drosophila Dorsal Closure

03.00 PM  Juan RIESGO-ESCOVAR
Regulation of Dorsal Closure in Drosophila Embryogenesis

03.30PM  Emmanuel FARGE
Mechanotransduction in Development, Conservation Across
Species and Evolutionary Incidences

04.00 – 4.30 PM  Tea break

04.00 – 4.30 PM  Elazar ZELZER
Muscle-Induced Mechanical Loads Regulate Key Aspects of
Skeletogenesis

05.00 PM  Philip KELLER
Reconstructing Neural Development

05.30 PM  Carl-Philip HEISENBERG
Mechanical Forces Driving Zebrafish Epiboly

06.00 PM  Karuna SAMPATH
Dynamic Microtubules at the Vegetal Cortex of Early Zebrafish
Embryos

Closing ceremony
G.V. Shivashankar (Conference Co-Chair)
Speaker Abstracts
Session I

Cell Mechanosensitivity and Integrin Adhesion
Cellular Mechanosensing at Multiple Levels

MICHAEL P. SHEETZ
Mechanobiology Institute and Department of Biological Sciences, NUS, Singapore
Department of Biological Sciences, Columbia University, New York, NY 10027

Control of cell morphology involves the integration of mechanical sensing and different types of cell motility to produce the desired shape of the organism\(^1\). In the case of cell-matrix interactions, it seems that there are several different levels of mechanosensing from an early testing of matrix stability, rigidity sensing, adhesion growth-maturation and finally, querying of the adhesions. Recent studies with supported bilayers containing lipid-bound RGD ligands, show that diffusive ligand binding causes clustering of αvβ3 integrins that stimulates actin polymerization and contraction\(^2\). If contraction is to a barrier in the bilayer, then further actin assembly is stimulated until the cell spreading removes the folds in the membrane and activates contraction to sense rigidity\(^3\). The process of rigidity sensing involves pulling to a constant displacement of about 130 nm\(^4\) in a local region of the cell. Once a rigid surface is sensed, the cell will assemble a focal adhesion through a force-dependent process. In mature adhesions, the stretching of proteins can unveil binding sites such as the stretching of talin causing the increased binding of vinculin\(^6\). Recent findings show that talin is stretched by 200-300 nm and relaxed multiple times in vivo with a stochastic period of 6-16s\(^7\). If the active head of vinculin is expressed, then the stretching is increased to about 400 nm and the oscillations in length are damped. These findings indicate that it is not a single stretch but perhaps the integral of many stretches that defines the cellular response to mechanical aspects of the environment. Because the assembly of talin in adhesion complexes depends upon the clustering of integrins, rigidity of the surface matrix, and formation of a mature adhesion, the integrated signal from a surface will be a complex function of both the chemical nature of the matrix, rigidity of the matrix and the level of cell motility.


Website
http://mbi.nus.edu.sg/michael-sheetz/
Mechano-regulation of Integrin Adhesion Dynamics

Benjamin Geiger¹, Israel Patla², Nadav Elad², Tova Volberg¹, Haguy Wolfenson⁵, Fabian Anderegg¹, Yoav Henis⁵, Joachim Spatz³, Reinhard Faessler⁴ and Ohad Medalia²

¹Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel;
²Department of Life Sciences, Ben Gurion University, Beer-Sheva, Israel;
³Department for New Materials and Biosystems, Max-Planck-Institute for Intelligent Systems, Stuttgart, Germany;
⁴Max-Planck Institute of Biochemistry, Martinsried, Germany;
⁵Department of Neurobiology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Cell adhesion to the extracellular matrix is a complex process, regulated by multiple genes, at multiple levels. Cells can differentially sense and respond to diverse chemical signals, defined by the molecular composition of the extracellular matrix, as well as to a variety of physical cues, including external or cytoskeleton-generated forces, surface rigidity, geometry and nano-topography. These structural and signaling activities are executed by multi-molecular protein complexes, known collectively as the integrin adhesome, and consisting of specific receptor molecules, actin-associated molecules, adaptor proteins and signaling molecules. Collectively, these protein ensembles control and integrate the interactions of cells with their environment, and the signaling processes triggered at these adhesion sites (e.g. focal adhesions and podosomes), and regulate their effect on the cell’s behavior and fate. In this presentation, I will describe our attempts to unravel the molecular nano-architecture and functional organization of different types of integrin adhesions. I will particularly focus here on a combination of synthetic approaches for extracellular matrix engineering and advanced approaches for correlated microscopy, in which the same specimens are examined by fluorescence microscopy and by cryo-electron tomography. A critical feature of integrin adhesions; namely, their capacity to respond to chemical and physical environmental cues, will be described, and novel results, pointing to mechanical regulation of the molecular dynamics of the adhesion site, will be presented. The results obtained using these novel experimental strategies provide new insights into the structure, dynamics and function of focal adhesions of cultured cells, and elucidation of their molecular organization and roles in matrix adhesion, surface sensing, cytoskeletal assembly and cell migration.

Website
http://www.weizmann.ac.il/mcb/Geiger/pages/contact.html
Forces Across Cell Adhesion Receptors

MARTIN A. SCHWARTZ
Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908, USA

Cell adhesion receptors are key integrators of cell adhesion, mechanical forces and cellular regulation. This 3-way connection is critical to morphogenesis, where high precise, 3-dimensional multicellular structures are required. Cell adhesion receptors play a key role in converting mechanical forces to biochemical signals, a process termed mechanotransduction. We have developed a new tool to understand mechanotransduction: a FRET module that can be used to measure physical force across specific molecules. I will present our latest results on the use of this method and our current understanding of how cell adhesion receptors mediate responses of cells to mechanical force.

Website
http://bme.virginia.edu/people/schwartz.php
Cell-Matrix Adhesion-Dependent Assembly of the Actin Cytoskeleton

ALEXANDER D. BERSHADSKY (1,2),
Tom Shemesh (1), Yee Han Tee (2), Michael M. Kozlov (3), Alexandra Lichtenstein (1), Masha Prager-Khoutorsky (1), Ramaswamy Krishnan (4), Benjamin Geiger (1)

(1) Weizmann Institute of Science, Rehovot, Israel
(2) Mechanobiology institute, National University of Singapore, Singapore
(3) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
(4) Harvard School of Public Health, Boston, MA, USA

Well-organized system of focal adhesion (FA)-associated actomyosin stress fibers (SFs) plays a major role in the maintenance of cell shape, generation of cell traction forces, and regulation of cell migration. Fibroblasts attached to flat rigid isotropic substrate spread first isotropically and form isotropic system of radial and tangential SFs associated with the radial FAs. Eventually, however, these cells undergo polarization manifested by cell elongation, segregation of cell periphery into active (lamellipodia forming) and stable regions, and formation of the array of SFs oriented along the long axis of the cell. Here we show that orientation of FAs and organization of the array of parallel SFs precedes cell elongation and formation of active and stable cell edges. Moreover, parallel orientation of SFs-FAs develops finally even in the cells, elongation of which was artificially suppressed by plating on circular adhesive islands. We demonstrate that fibroblast polarization and formation of SF arrays depends on FA mechanosensitivity and does not occur on compliant substrates. A unifying physical model explaining the main aspects of the SF array organization is presented. siRNA-mediated knockdown of 85 human protein tyrosine kinases (PTKs) induced distinct alterations in the cell polarization response, as well as diverse changes in cell traction force generation and FA formation. Remarkably, changes in rigidity-dependent traction force development, or FA mechanosensing, were consistently accompanied by abnormalities in the cell polarization response. We propose that the different stages of cell polarization are regulated via multiple, PTK-dependent molecular checkpoints that jointly control cell contractility and FA-mediated mechanosensing.

Website
http://mbi.nus.edu.sg/alexander-bershadsky/
A Materials Approach to Studying Mechanotransduction

CHRISTOPHER S. CHEN
Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA.

Adhesion of cells to materials, natural or synthetic, appears to be a central regulator of cellular functions, including proliferation, differentiation, migration, and suicide. Adhesion involves not only receptor binding, but also changes in cell shape and the generation of mechanical stresses at these adhesions. Using microengineered materials as substrates for cell adhesion, we explore the relative contributions of these different aspects of adhesion to regulating cell function. In this presentation, we will present our recent progress on using a variety of engineered materials to study the mechanics of cell adhesion, and show how the interactions between cell shape, mechanical forces, and adhesion can impact cell function. These studies highlight the importance of novel engineering and materials approaches to inform our basic understanding of how cells respond to their environment.

Website
http://www.seas.upenn.edu/~chenlab/
A Large Scale Mechanosensing Mechanism to Explain the Cell Response to the Stiffness of the Substrate

Léa Trichet¹, Jimmy Le Digabel¹, Rhoda J. Hawkins², Sri Ram Krishna Vedula³, Claire Ribault¹, Raphaël Voituriez², Pascal Hersen¹, ³ and Benoît Ladoux¹, ³

¹ Laboratoire Matière et Systèmes Complexes (MSC), CNRS UMR 7057 & Université Paris Diderot, Paris, France ; ² CNRS UMR 7600 & Université Pierre et Marie Curie (UPMC), Paris, France ; ³ Mechanobiology Institute (MBI), National University of Singapore, Singapore.

By affecting the dynamics of adhesion signaling and cytoskeletal organization, mechanical forces play a key role in various cellular processes, from propelling cell migration to mediating interactions between neighboring cells. As they anchor and pull on their surroundings, adhering cells actively probe the stiffness of their environment. Current understanding is that traction forces exerted by cells arise mainly at focal adhesions whose size seems to be regulated to maintain a constant stress. Here I will present our results that are contrary to this assumption. We show by direct measurements that the build-up of traction forces is faster for larger substrate stiffness and that the stress measured at adhesion sites depends on substrate rigidity. I will then present our phenomenological model based on active gel theory which suggests that rigidity-sensing is mediated by a large scale mechanism originating in the cytoskeleton, instead of a local one.

To assess the spatial resolution of cellular rigidity sensing, we develop composite micropillar substrates with alternative areas made soft and stiff pillars. We show that a large scale mechanosensing mechanism could explain the adaptive response of cell migration to stiffness gradients. In response to a step boundary in rigidity, we observe, not only that cells migrate preferentially towards stiffer substrates, but also that this response is optimal in a narrow range of rigidities. Taken together, these findings lead to new insights into the regulation of cell response to external mechanical cues and provide evidence for a cytoskeleton-based rigidity sensing mechanism.

Website
http://mbi.nus.edu.sg/benoit-ladoux/
Insights from Proteomic Analyses of Adhesion Signalling

MARTIN J. HUMPHRIES
Faculty of Life Sciences, The University of Manchester, Manchester, UK.

Multicellular life requires the formation of extracellular matrices and the sensing of the cell environment through regulated adhesive interactions. By coupling the binding of extracellular adhesion proteins to the assembly of intracellular cytoskeletal and signalling complexes, integrin receptors mediate the bidirectional transmission of mechanical force and biochemical signals across the plasma membrane. Integrin-based adhesion is highly dynamic, as cells must rapidly respond to changes in their environment by altering their migratory properties, gene expression profile and proliferation state. A detailed, integrative view of the dynamics of adhesion complexes would provide insight into the molecular mechanisms that control cell morphology, movement, survival and differentiation, but, as with other membrane receptor–associated signalling complexes, integrin adhesion complexes have been refractory to isolation due to their instability and inaccessibility. A literature-curated model for the composition of adhesion complexes has revealed massive complexity (1), but despite decades of work, the global composition and mechanisms of regulation of integrin-associated protein complexes are relatively poorly understood. We reasoned that there was a need for technologies that enable systematic, proteomic analysis, and accordingly we have developed a methodology for the affinity isolation and mass spectrometric analysis of integrin adhesion complexes (2). In follow-up studies to this original publication, the isolation of stabilised complexes associated with multiple integrin receptor–ligand pairs, and detailed quantitative analyses of their composition at multiple time points and in different receptor activation states, have been carried out. Our analyses have defined temporal profiles of integrin-associated protein complexes during the initial stages of cell adhesion, and compared the complexes that are assembled by integrins occupied either by ligands or by monoclonal antibodies that freeze receptor conformation in different states of activation. Hierarchical clustering and protein interaction network analyses reveals distinct dynamics of protein modules relevant to cell adhesion processes. Although we should not underestimate the scale of the task, the development of this workflow now permits the molecular dynamics of adhesion complexes to be measured directly and presents an entry point for quantitative, systems-level analyses of adhesion signalling in health and disease.


Website
http://www.ls.manchester.ac.uk/people/profile/index.aspx?PersonID=849
Session II
Host-Microbe Interactions and Bacterial Collective Behavior
A 3-D Structure of the E.coli Chromosome?

Sankar Adhya
Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892–4264, USA

Bacterial nucleoid organization is believed to have minimal influence on the global transcription program. Using an altered bacterial histone-like protein, HUalpha, we show that reorganization of the nucleoid configuration can dynamically modulate the cellular transcription pattern. The mutant protein transformed the loosely packed nucleoid into a densely condensed structure. The nucleoid compaction, coupled with increased global DNA supercoiling, generated radical changes in the morphology, physiology, and metabolism of wild-type K-12 Escherichia coli. Many constitutive housekeeping genes involved in nutrient utilization were repressed, whereas many quiescent genes associated with virulence were activated in the mutant. We propose that, as in eukaryotes, the nucleoid architecture dictates the global transcription profile and, consequently, the behavior pattern in bacteria. We have evidence to show that the E. coli chromosome has a 3-D structure that likely guides the transcription profile.

Website
http://ccr.cancer.gov/staff/staff.asp?profileid=5783
Mechano-Microbiology: Surface Sensing in P. Aeruginosa
Motility and Virulence

ZEMER GITAI
Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA

The proteins of the bacterial cytoskeleton have emerged as central regulators of bacterial growth and division. Recently, we have discovered that cytoskeletal proteins serve additional functions in bacteria. For example, the bacterial actin homolog, MreB, regulates the localization and assembly of the type IV pilus virulence factors in Pseudomonas aeruginosa. This regulation of pilus assembly occurs at multiple biochemical levels and depends on the stiffness of their surface substrates, indicating that P. aeruginosa can sense its mechanical environment. Further studies demonstrate that stiff surfaces influence both gene expression and the mechanics of P. aeruginosa motility. Together, these results suggest that bacteria can sense their mechanical environments and use mechanical information to regulate their development and virulence.

Website
http://www.molbio1.princeton.edu/labs/gitai/
The Biofilm Life Cycle

Yehuda Cohen

Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore

The bacterial life style involves the transformation from single free living cells to the development of biofilm communities composed of several different physiological groups in close proximity to each other, embedded in an exo-polymeric matrix. The biofilm is the default mode of microbial life, witnessed in the earliest microfossils dated over 3.6 billion years ago. All biofilms are surface interactions and require bacteria to detect surfaces, orient towards surfaces and initiate the transformation into a biofilm. An early example of this transformation was described by Mel Simon and others in the 1980s in Vibrio parahaemolyticus. Since then Pseudomonas aeruginosa has served as a model for our understanding of microbial biofilms. Yet the biofilm mode of life is much more prevalent across a large spectrum of microorganisms. In all cases cell-cell contact is key for biofilm development. Biofilm development is controlled by a series of internal signalling compounds, in a manner similar to that of the differentiation process of tissue cultures, as well as a range of external environmental diffusion clues. The latter are unique for microbial biofilm development, and the spectrum of biofilm differentiation processes is significantly broader than witnessed in tissue cultures. Hence, there are many exciting and informative similarities and differences of the mechanobiology of tissue differentiation to that of the biofilm life cycle. Cross talk across these rapidly developing disciplines is likely to enhance our understanding of both prokaryotic and eukaryotic multicellular differentiation and development systems.

Website
http://www.ntu.edu.sg/ohr/PIC2011/Pages/ProfYehuda.aspx
Viruses have evolved numerous mechanisms to hijack and subvert the different cellular systems of their host to facilitate their entry, replication and spread. We use a combination of biochemical and imaging approaches to study vaccinia virus as model system to obtain molecular insights into signalling, cytoskeletal dynamics and microtubule-based transport. *During infection, newly assembled* virus particles, travel on microtubules from their peri-nuclear site of assembly to the plasma membrane by recruiting kinesin-1. Kinesin-1 is recruited by a bi-partite tryptophan based motif in the integral viral membrane protein A36, which interacts with the kinesin-1 light chain. The virus also enhances its ability to reach and fuse with the plasma membrane by increasing microtubule dynamics and modulating the organization of the cortical actin cytoskeleton by F11-mediated inhibition of RhoA signalling to mDia. Virus particles, which fuse with the plasma membrane and remain attached to the cell activate Src and Abl family kinases. This activation leads to phosphorylation of tyrosine 112 and 132 of A36, which results in the recruitment of a signalling network consisting of Grb2, Nck, WIP and N-WASP that stimulates Arp2/3 complex dependent actin-based motility of virus particles and enhances the spread of infection.

Website
http://lrilabs.org/research/loc/london/lifch/waym/?view=LRI&source=research_portfolio
Cryo-Electron Tomography of Trypanosome Flagellum

Cynthia He

Department of Biological Sciences, National University of Singapore, Singapore

Trypanosoma brucei is a parasitic protozoan that causes human African sleeping sickness. It contains a single flagellum essential for cell motility and viability. In addition to a 9+2 microtubular axoneme, the flagellum also contains a paraflagellar rod (PFR) linked to the axoneme by connecting proteins. Using cryo-electron tomography (cryo-ET), we showed the structure of the flagellum in three bending states. We found that the PFR has a deformable crystal lattice, whose unit cell diagonal repeats every 56 nm along the length of the straight axoneme, matching the spacing of the connecting proteins. During flagellar bending, the PFR crystal unit cell lengths remain constant while the interaxial angles vary, similar to the action of a jackscrew. The beating of the axoneme drives this expansion and compression of the PFR lattice through the connecting proteins. We propose that the PFR modifies the in-plane axoneme motion to produce the characteristic trypanosome bihelical motility as captured by high-speed light microscope videography.

Website
http://www.dbs.nus.edu.sg/staff/cynthia.htm
Keynote Lecture 3
Biology and Physics of von Willebrand Factor Concatamers

Timothy A. Springer

Department of Pathology, Harvard Medical School, Boston, MA, USA and Immune Disease Institute, Program in Cellular and Molecular Medicine at Children’s Hospital, Boston, MA, USA

Structural specializations enable von Willebrand factor (VWF) to assemble during biosynthesis into helical tubules in Weibel-Palade bodies (WPB). Specializations include a pH-regulated dimeric bouquet formed by the C-terminal half of VWF and helical assembly guided by the N-terminal half that templates inter-dimer disulfide bridges. Orderly assembly and storage of ultra-long concatamers in helical tubules, without crosslinking of neighboring tubules, enables unfurling during secretion without entanglement. Length regulation occurs post-secretion, by hydrodynamic force-regulated unfolding of the VWF A2 domain, and its cleavage by the plasma protease ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13). VWF is longest at its site of secretion, where its hemostatic function is most important. Moreover, elongational hydrodynamic forces on VWF are strongest just where needed, when bound to the vessel wall, or in elongational flow in the circulation at sites of vessel rupture or vasoconstriction in hemostasis. Elongational forces regulate hemostasis by activating binding of the A1 domain to platelet GPlba, and over longer time periods, regulate VWF length by unfolding of the A2 domain for cleavage by ADAMTS13. Recent structures of A2 and single molecule measurements of A2 unfolding and cleavage by ADAMTS13 illuminate the mechanisms of VWF length regulation. Single molecule studies on the A1-GPlb receptor-ligand bond demonstrate a specialized flex-bond that enhances resistance to the strong hydrodynamic forces experienced at sites of hemorrhage.

Website
http://labs idi.harvard.edu/springer/
Session III
Mechanisms of Cadherin Adhesion
Structural Biology of Cadherin-mediated Cell-cell Adhesion

Lawrence Shapiro

Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA

The classical cadherins mediate calcium-dependent intercellular adhesion among cells to specify the organization of tissues. Structural studies using X-ray crystallography, with concomitant functional studies, have revealed the molecular underpinnings of their adhesive interaction and specificity, and show how they self-assemble to form the intercellular junctions that underlie stable adhesion between cells.

Website
http://www.shapirolab.org/
Physical Principles of Cadherin-mediated Cell-cell Adhesion

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A variety of cell-cell adhesion processes are mediated by the (trans) dimerization of cadherin proteins presented from apposing cell surfaces. Simulations carried out at different levels of granularity will be used to describe molecular design principles that lead to trans dimerization and to establish a correlation between measured binding affinities at the molecular level and cell-cell adhesive specificity. Following cell-cell contact, cadherin dimers cluster together to form cell-cell junctions. Since such clustering does not occur in the absence of cell-cell contact, there is a necessary coupling between trans and lateral (cis) interactions. The molecular mechanisms that underlies this coupling will be described. In addition to the insights that will be discussed in the context of cadherin function, general principles that may underlie the molecular basis of many cell-cell adhesion processes will be proposed.

Website
http://cpmcnet.columbia.edu/dept/gsas/biochem/faculty/honig.html
Diversity of Intercadherin Interactions in Adherens Junctions

SERGEY TROYANOVSKY

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Live imaging experiments showed that spot-like adherens junctions are continuously produced at the basal edge of the lateral surface of epithelial A431 cells. Then, the assembled junctions move apically. After reaching the apical surface these junctions fuse with much larger and less mobile apical adherens junctions. The latter junctions, in contrast to spot-like lateral junctions, co-recruit actin-binding proteins, such as vinculin, VASP and afadin, as well as another transmembrane protein, nectin-2. To understand the role of extracellular and intracellular cadherin regions in this cycle, different binding interfaces of cadherin ectodomain were modified in a way increasing or decreasing their binding strength. Results showed that the crucial event in junction formation is trans strand-swapping. The resulting adhesive dimers can be organized into junctions by a combination of their direct cis-interactions via ectodomains and by intracellular catenin-mediated interactions. The latter interactions are apparently different in apical and lateral adherens junctions.

Website
https://fsmweb.northwestern.edu/faculty/FacultyProfile.cfm?xid=17023
Control of Adhesive Strength in Cadherin-mediated Adhesion

JEAN-PAUL THIERY¹,
Chu, Y.S.¹, Thomas, W.², Martinez-Rico, C.², Mege, R.M.³, Viasnoff, V.⁴,⁵, Engl, W.⁴, Bershadsky, A.⁴,⁶ and Dufour, S.²


Cell adhesion is a primary process in development which plays a major role in morphogenesis. Defect in cell adhesion can lead to embryonic lethality or to severe malformation during histogenesis. Adhesion processes are also very critical post-natally. Any defect in cell adhesion can have severe consequences on tissue homeostasis, blood clotting, immune response and tissue repair. The role of cell adhesion is also well established in tumor progression particularly during metastasis.

To unravel mechanisms controlling the strength of adhesion we used sarcoma S180 cell line which lacks cadherin-mediated intercellular adhesive properties. Stable transfectants of S180 cells, expressing either type I N-cadherin or type II cadherin-7, were analyzed in vivo and in vitro cell motility assays. A dual pipette assay was modified to measure forces required to separate S180 cell doublets expressing different levels of type 1 or type 2-cadherins. The force required to separate E-cadherin expressing doublets was critically depending on the duration of contact and levels of expression. An initial adhesion not exceeding several nanonewtons is reinforced only following cortical actin polymerization. This mechanism is dependent on CDC42 and Rac activation. Type II cadherin-7 or -11 is significantly less adhesive than E or N-cadherin. These results suggest that in vivo, cadherin-7 or -11 mediates transient adhesion, which favors motility, and invasion, while E or N-cadherin mediates stable contacts. We have explored the mechanism of adhesion strengthening using our dual pipette assay and a flow chamber. Our results strongly suggest that several cadherin associated proteins are critical for the reinforcement of adhesion. In addition the cortical actin cytoskeleton plays a major role in this process. Cell separation may be accomplished mainly by peeling the cortical actin cytoskeleton from the plasma membrane in area of cells in contact. We will conclude this presentation by discussing recent data on mechanosensing mediated by alpha-catenin in regulating adhesion strengthening.

Website
Polarized Contractility of Adherens Junctions Leads to Neural Tube Closure

Masatoshi Takeichi
and Tamako Nishimura

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Closing of the neural tube is a critical step for embryonic morphogenesis, and its failure causes serious birth defects. Neural tube closure is achieved by the bending of the neural plate along the anterior-posterior axis of an embryo. This bending depends on the constriction of the apical surface of individual neuroepithelial cells, particularly at the median hinge point (MHP). The epithelial apical constriction is induced by actomyosin dependent-contraction of adherens junction (AJs), which is located near the apical side of the cells. To achieve the “polarized” bending of an epithelial cell sheet, however, AJ contraction also should be polarized; otherwise, the sheet may bend radially. We actually found that, at the apical plane of bending neural plates, the AJ-associated actomyosin is activated selectively along the mediolateral axis of the plate (1), suggesting that this phenomenon may be important for the polarized bending of the neural plate. We have now revealed that the local activation of AJ-associated actomyosin is induced by a set of PCP signaling molecules, and the blockage of this signaling process inhibits neural tube closure. We also analyzed the behavior of individual cells during neural plate bending, and found that the polarized contraction of AJs leads to the convergent relocation of the apical domain of plate cells toward the midline, which results in the mediolateral apical constriction of the neural plate. These results suggest that the PCP-dependent contraction of AJs plays a major role in neural tube closure.

Reference

Website
Keynote Lecture 4
Rho GTPases and the Morphogenesis and Migration of Epithelial Cells

Alan Hall,
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Memorial Sloan-Kettering Cancer Center, Cell Biology Program, New York, NY 10065, USA

Rho GTPases control signal transduction pathways linking plasma membrane receptors to the assembly of F-actin and associated cell-matrix and cell-cell adhesion complexes. In addition, they promote a diverse set of other cellular activities, including changes in gene transcription, the establishment of cell polarity, cell cycle progression, the organization and dynamics of the microtubule cytoskeleton and the activation of a variety of enzymes including PI 3-kinase and NADPH oxidases. The actin and microtubule cytoskeletons play a central role in driving many of the dynamic aspects of cell behavior. Our particular focus is the molecular mechanisms by which Rho GTPases regulate cell migration and cell morphogenesis in epithelial cells. We have been using two human cell lines, 16HBE derived from airway epithelium after immortalization with T antigen, and Caco-2, derived from a colorectal cancer, but lacking mutations associated with more aggressive tumors, such as Ras or PI3K. 16HBE cells establish apical basal polarity and tight junctions when grown in 2D yet show remarkable features of collective migration. Caco-2 form polarized cysts when grown in 3D, with many characteristics of normal, differentiated colonic epithelium. We are exploring the role of Rho GTPases in these systems. The disruption of normal tissue architecture and the appearance of inappropriate migratory activity are two defining characteristics associated with cancer progression towards an invasive and metastatic phenotype. Our long-term goal is to identify the mechanisms that drive these changes.

Website
Session IV

Cytoskeleton, Signaling and Cell-Cell Junctions
Translating Time into Space: Regulating the Rho GTPase Cycle at the Epithelial Zonula Adherens

Aparna Ratheesh¹, Guillermo Gomez¹, Rashmi Priya¹, Eva M. Kovacs¹, Nicholas Brown², Anna Akhmanova³, Samantha J. Stehbens¹, and Alpha S. Yap¹

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The epithelial zonula adherens (ZA) is a specialized adhesive junction that supports tissue cohesion and morphogenesis. At the ZA, E-cadherin adhesion receptors are functionally coupled to the actin cytoskeleton to form a dynamic, integrated cellular system. Cortical signals play key roles in this cooperation. In particular, the ZA is a cortical zone where Rho concentrates at steady-state in established epithelial monolayers. We now identify a novel cellular mechanism that coordinates both activation (GEF) and inactivation (GAP) limbs of the Rho GTPase cycle to maintain the Rho zone of the ZA. We found that Rho signaling at the ZA depends on dynamic microtubules. This involves the microtubule-dependent localization of the centralspindlin (CS) complex. Best understood for its role in cytokinesis, we now report that CS is also a junctional component that localizes specifically to the ZA of interphase epithelial monolayers. CS activates Rho signaling by recruiting the RhoGEF, Ect2, to junctions. This ultimately supports ZA integrity by signaling to myosin IIA. Centralspindlin further promotes the junctional Rho zone by inhibiting the junctional localization of p190 RhoGAP B, thereby preventing premature inactivation of Rho. CS binds to the E-cadherin/catenin complex through α-catenin; consistent with this, α-catenin is necessary for cortical retention of Ect2 and for Rho signaling at the ZA. We thus identify the CS complex as a novel component of the ZA, which exerts a conserved, extramitotic function to support ZA integrity by regulating junctional Rho.

Website
Rho GTPases and the Regulation of Epithelial Contraction and Differentiation

Vania Braga

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Cell-cell adhesion and contraction play an essential role in the maintenance of geometric shape (2D topology) and polarization of epithelial cells (3D shape). However, the molecular regulation of contraction during epithelial polarization and geometric cell shape acquisition is not clear. Induction of cell-cell adhesion, we find that human keratinocytes acquire specific geometric shapes favouring hexagons, by re-modelling junction length/orientation and thus neighbour allocation. Acquisition of geometric shape correlates temporally with epithelial polarization, as shown by an increase in lateral height. ROCK1 and ROCK2 are important regulators of myosin II contraction, but their specific role in epithelial cell shape has not been addressed. Depletion of ROCK proteins interferes with the correct proportion of hexagonal cell shapes and full elongation of the lateral domain. Interestingly, ROCK proteins are not essential for maintenance of circumferential thin bundles, the main contractile epithelial F-actin. Instead, ROCK1 or ROCK2 regulates thin bundle contraction and positioning along the lateral domain, an important event for the stabilization of the elongating lateral domain. Mechanistically, E-cadherin clustering specifically leads to ROCK1/ROCK2-dependent inactivation of MYPT and up-regulation of MRLC phosphorylation. We will discuss the significance and temporal correlation of these events with keratinocyte polarization and geometric shape acquisition.

Website
https://www1.imperial.ac.uk/medicine/people/v.braga/
Tiam1 Acts with the PAR Complex to Control Talin-mediated Rac1 Activation During Polarized Cell Migration

Kozo Kaibuchi

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Directional cell migration is required for various physiological processes such as embryonic development, angiogenesis, wound healing, and tumor invasion. Migrating cells acquire ‘front-rear’ polarity along the directional axis, with signaling molecules, adhesions and the cytoskeleton distributed asymmetrically. Adhesion molecules such as integrins play a critical role in establishing cell polarity. The binding of integrins to the extracellular matrix (ECM) activates intracellular signaling pathways that regulate migration (outside-in signaling), whilst the affinity of integrins for the ECM can be regulated by signals within cells (inside-out signaling). Talin is a key participant in both outside-in and inside-out signaling. Talin associates with the cytoplasmic region of integrin β, and increases binding affinities of integrins for the ECM. Furthermore, talin functions as a molecular bridge to link integrins with various signaling molecules. Among these, the Rho family GTPases (especially Rac) and the PAR complex (composed of PAR3, PAR6 and aPKC) cooperatively play key roles in polarized cell migration.

We previously found that PAR3 interacts with Tiam1, a Rac1-specific GEF, and further forms a complex with aPKC, PAR6 and Cdc42, thereby mediating Cdc42-induced Rac1 activation (Nishimura et al., Nat Cell Biol 2005). Tiam1, with PAR3, controls front-rear polarity of migrating cells (Pegtel et al., Curr Biol 2007; Nakayama et al., Dev Cell 2008). Although several Rac GEFs including Tiam1 are implicated in Rac1 activation downstream of integrins, how integrins activate Rac1 during cell adhesion, polarization and migration remains largely unknown. How Tiam1 and PAR3 regulate polarized migration also remains elusive.

We show here that Tiam1 interacts directly with talin. In migrating cells, Tiam1 accumulates at focal adhesions in a manner dependent on talin and the PAR complex. The interaction of talin with Tiam1, and the PAR complex, are required for adhesion-induced Rac1 activation, cell spreading, and migration toward integrin substrates. Furthermore, Tiam1 acts with talin to regulate focal adhesion turnover for polarized migration.

Website
http://www.med.nagoya-u.ac.jp/Yakuri/index_english.htm
Contribution of Cadherin Anchoring and Force Transduction to Cell-Cell Contact Maturation and Cell Migration

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Institut du Fer à Moulin, Université Pierre et Marie Curie, Paris, France

During normal development and in pathological manifestations such as cancer, opportunistic cell-cell contacts either drive the re-enforcement of these contacts leading to the formation of stable intercellular junctions or, at the opposite, maintain cell contact instability leading to cell-cell intercalation or even long distance cell migration. They however involve common coordinated processes among which plasma membrane adhesion to the surrounding environment and cytoskeleton remodeling are relying on cadherin adhesion receptors mobilization and regulation of actin dynamics, respectively. Cadherins form homophilic trans-dimers with identical receptors in the membrane of adjacent cells, while they anchor intracellularly to actin filaments via catenins. These cadherin adhesions allow the efficient transmission of traction forces generated by the cytoskeleton. In turn, the forces applied by cytoskeleton-associated motors on cadherin assemblies direct tension-dependent strengthening of cell-cell contacts. In elongated axons, this mechano-transduction is at the basis of the mechanisms of migration of the growth cone.

Another key actor of this cellular plasticity is the microtubule networks (MTs). However, while the cadherin - actin functional crosstalk has been well studied, the relationship between MTs, cadherin and actin is still unclear and subject to controversy. We will report on our recent studies regarding the connection between N-cadherin and MTs during stable cell-cell contact formation as well as during cadherin-induced migration, i.e. neurite outgrowth. We analyzed the effect of cadherin engagement on MTs dynamics in cells spread on N-cadherin substrates mimicking cell-cell contacts. We observed that in both situations (stables contacts and cell migration) newly formed MTs were recruited by their +tips at the adhesion sites. In C2 myogenic cells that form stable intercellular junctions, N-cadherin adhesion had an inhibitory effect on the penetration of MT +tips in adhesion areas, on the recruitment of +tips proteins of the CLIP and EB families, as well as on the overall MT dynamics. In contrast, N-cadherin engagement had a stimulatory effect on MT dynamics in neurites of primary hippocampal neurons. The opposite effects were alleviated by actin networks depolymerization. We are currently investigating the influence of N-cadherin / actin decoupling on MT dynamics and the impact of MT dynamics inhibition on both cell-cell contact reinforcement and induction of cell migration stimulated by N-cadherin adhesion.

Website
http://www.u839.idf.inserm.fr/
Actin Worms and Myosin Networks: Towards an Integrated View of the Actin Cortex

Anoop V. Cherian, Christoph Klingner, Philipp M. Diesinger, Roland Aufschnaiter, Thomas Keil, Nicola Maghelli, Gisela Beck, Iva Tolic-Norrelykke, Mark Bathe and Roland Wedlich-Söldner

Max Planck Institute of Biochemistry, Cellular Dynamics and Cell patterning, Martinsried, Germany.

Most mammalian cells exhibit actin-based protrusions on their non-adherent surfaces. Microvilli (MV) have mainly been characterized in epithelial monolayers, where they form dense apical arrays. MV-like protrusions of varying density and shape have also been reported in many non-epithelial cell types. However, a detailed study of their distribution, origin and in particular their dynamics is still lacking. Here we demonstrate that non-confluent epithelial cells exhibit characteristic actin bundles on their apical surface that are distinct from classical MV in appearance and dynamics. We have termed these bundles “actin worms” to reflect the bending undulations that are characteristic of their shape and movement. These actin worms exhibit complex dynamical rearrangements and are coupled within a structurally isotropic network that spans the apical cell surface. Both the dynamics and connectivity of actin worms depend on the activity of type II myosins. Specifically, we found that in cells exhibiting actin worms, myosin II forms two-dimensional networks of filaments covering the apical cell surface. Further, actin worms and myosin filaments exhibit coordinated oscillatory movements and are under mechanical tension. Finally, we demonstrate that actin worms and apical acto-myosin networks develop during wound healing and upon induction of EMT. Taken together, these observations suggest that a dynamic acto-myosin network laterally organizes the apical surface of epithelial cells and provides mechanical support during cell migration and morphogenesis.

Website
Roles of Nectins in Heterotypic Cell Adhesion

YOSHIKI TAKAI

Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine

Organs and tissues in mammals are composed of different types of cells which homotypically and heterotypically adhere to each other mainly by adherens junctions (AJs). A major cell adhesion molecule at AJs is cadherin which comprises a family with many members. Each member is expressed in specific cell types and trans-interacts only homophilically. However, the mechanism of the heterotypic adhesion observed in organs and tissues cannot be explained solely by this property of cadherins. My laboratory found another cell adhesion molecule nectin which comprises a family with four members. The members of nectin trans-interact both homophilically and heterophilically and their heterophilic trans-interactions are much stronger than their homophilic trans-interactions. Because of this property, nectins play roles in both homotypic and heterotypic cell adhesions cooperatively with or independently of cadherins. For instance, in epithelial cells of the small intestine and the kidney, nectins first initiate cell adhesion and then recruit E-cadherin to the nectin-based cell adhesion site to establish AJs. In the hippocampus of the brain, nectin-1 and -3 are localized at axon terminals and dendrites, respectively, and their trans-interaction first initiates their adhesion and then establishes synapses cooperatively with N-cadherin. In the testis, nectin-2 and -3 are specifically expressed in Sertoli cells and spermatids, respectively, and their trans-interaction is involved in their adhesion and differentiation of spermatids independently of any members of the cadherin family. In the auditory epithelium of the inner ear, hair cells are interdigitated by supporting cells, causing the formation of a checkerboard-like pattern. Nectin-1 and -3 are specifically expressed in hair and supporting cells, respectively, and their trans-interaction is involved in the formation of this unique cellular pattern. In this meeting, the roles of nectins in heterotypic cell adhesion will be described.

Website
http://www.med.kobe-u.ac.jp/mcb/index.html
TMP-Tag: A Chemical Surrogate to the Fluorescent Proteins for Live Cell Imaging

Virginia W. Cornish

Department of Chemistry, Columbia University

The fluorescent proteins revolutionized our ability to study protein function directly in the cell by enabling individual proteins to be selectively labeled through genetic encoding of a fluorescent tag. As researchers seek to make increasingly sophisticated dynamic measurements of protein function in the cell to unravel molecular mechanism, we designed a chemical tag to combine the advantages of genetic encoding with a modular organic fluorophore. With TMP-tag, the protein of interest is tagged with E. coli dihydrofolate reductase, which can subsequently be labeled with a cell permeable trimethoprim-fluorophore conjugate. Here we demonstrate that TMP-tag is a robust cellular reagent. We present recent results exploiting the modular nature of the chemical tag to generate TMP-tags for specific applications in single-molecule and super-resolution imaging.

Website
http://asp.cpmc.columbia.edu/pharm/pharmfac/profile_list.asp?ItemNumber=40
Session V

Cell Force Response and Collective Cell Movements
Collective Cell Guidance by Cooperative Intercellular Force

JEFFREY J. FREDBERG

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Cells comprising a tissue migrate as part of a collective. How collective processes are coordinated over large multi-cellular assemblies has remained unclear, however, because mechanical stresses exerted at cell–cell junctions have not been accessible experimentally. We report here maps of these stresses within and between cells comprising a monolayer. Within the cell sheet there arise unanticipated fluctuations of mechanical stress that are severe, emerge spontaneously, and ripple across the monolayer. Within that stress landscape, local cellular migrations follow local orientations of maximal principal stress. Migrations of both endothelial and epithelial monolayers conform to this behaviour, as do breast cancer cell lines before but not after the epithelial-mesenchymal transition. Collective migration in these diverse systems is seen to be governed by a simple but unifying physiological principle: neighbouring cells join forces to transmit appreciable normal stress across the cell–cell junction, but migrate along orientations of minimal intercellular shear stress.

Website
http://www.hsph.harvard.edu/fredberglab/index.html
Collective Migration of Epithelial Cells: the Role of Leader Cells

M. Reffay, L. Petitjean, O. Cochet, J. Camonis, M. C. Parrini, A. Buguin, B. Ladoux, and Pascal Silberzan

Institut Curie, Paris, France

Classical wounding experiments in confluent monolayers consist in mechanically scratching them. To overcome some of the ambiguities inherent to this technique, we have recently designed an original microfabrication-based technique to generate «model wounds» by using a micro-stencil that masks portions of the surface during the growth of a monolayer. Removing the stencil after epithelial MDCK cells have reached confluence does not damage the border cells but triggers the collective motility of the epithelium while cells maintain strong adhesions between them.

By using particle image velocimetry (PIV), based on the correlations between successive images, we observe that this collective motility involves long-range coordinated displacements of large groups of actively migrating cells well within the monolayer. These observations are in sharp contrast with the behavior of fibroblasts for which mostly the border cells are affected.

In parallel, the leading edges of these monolayers roughen drastically and exhibit a strong directional fingering where the fingers are led by single “leader” cells that acquire a very particular phenotype (fibroblast-like at their front side and epithelial-like at their backside) although they were initially not discernable from the others. To investigate the mechanisms underlying these pluricellular structures, we have studied them both at large scale (population scale) and at the scale of the individual cells composing them. We have fully mapped the velocity field (intensity and orientation) in the fingers and quantified the orientation of the cells themselves, as well as their division axis and the position of their MTOC relatively to their nucleus. We find that all these directions align with the fingers but are described by different order parameters and kinetics.

The force distribution in the fingers was mapped using a microfabricated array of force sensors and correlated with the velocity distribution as well as with the activity of the GTPases known to be involved in migration, using FRET biosensors. These series of experiments, complemented by laser photo-ablation experiments, help to clarify the particular contribution of the leader in these structures and their interplay with the other cells of the fingers. Interestingly, similarly looking leader cells are found in a large number of different situations in morphogenesis or local invasion from tumors.

References


Website: http://umr168.curie.fr/en/profile/pascal-silberzan-001057
Mapping the Collective Migration of Epithelial Cells

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C.T. Lim1, 2,*

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2. Department of Bioengineering, National University of Singapore, Singapore
3. Paris-Diderot University, Paris, France

It is a well known fact that cells migrating in sheets or large cohorts tend to behave very differently from cells migrating individually. Indeed, the distinctive behavior of cells migrating in a collective manner underlies several important biological processes such as those found in developmental biology, wound healing and even metastasis. Though the factors determining the distinctive migratory characteristics of cells in a cohort is poorly understood; in vitro (e.g. traditional wound healing assay) and in vivo (e.g. imaging embryos) experiments suggest that intercellular adhesion, guidance from chemical cues and mechanical constraints of the extracellular matrix through which cells migrate play an important role. However, in such experiments, it is not only difficult to independently assess the contribution of physical constraints vis-à-vis guidance from chemical cues but also impossible to map the distribution of traction forces exerted by cells on the substrate. Here, using a simple and novel application of microcontact printing, we characterized the kinematic behavior of cell cohorts migrating under well defined geometrical constraints as well as mapped the distribution of traction forces in such cohorts.

Website
http://mbi.nus.edu.sg/lim-chwee-teck/
Multi-cell Interactions in Metastatic Cancer

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ROGER D. KAMM

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Metastatic cancer often involves a sequence of events: the separation of individual cells from the primary tumor, migration through host tissue under the action of biochemical gradients and physical factors such as interstitial flow, intravasation into the vascular system, extravasation at a remote site, and the colonization, growth and vascularization of a peripheral tumor. Each of these processes involves a complex set of signaling events among multiple cell types in a variety of microenvironmental settings. Studies have been performed using various designs of a microfluidic platform to simulate several stages of metastasis: epithelial-mesenchymal transition (EMT), migration through the extracellular matrix, angiogenesis, intravasation, and extravasation. Selected results will be presented addressing several of these phenomena. Studies of intravasation show how the presence of accessory cells (e.g., macrophages) appear to be necessary for vascular wall crossing. Extravasation, in contrast, appears to occur readily with no need for other cell types, and occurs soon after contact with the endothelial monolayer. Tumor vascularization is influenced by secreted factors from tumor cells and by local interstitial flows. Examples will focus on the critical role of mechano-biology in these behaviors.

Website
http://web.mit.edu/meche/mb/
Motility and Directed Migration of Zebrafish Primordial Germ Cells

EREZ RAZ

Institute of Cell Biology, ZMBE, Muenster, Germany

Chemokine-guided germ cell migration in zebrafish requires the function of the small Rho GTPases Rac1 and RhoA, as well as E-cadherin-mediated cell-cell adhesion. Using fluorescence resonance energy transfer we demonstrate that Rac1 and RhoA are activated in the cell front. At this location, Rac1 is responsible for the formation of actin-rich structures, and RhoA promotes retrograde actin flow. We propose that these actin-rich structures undergoing retrograde flow are essential for the generation of E-cadherin-mediated traction forces between the germ cells and the surrounding tissue and are therefore crucial for cell motility in vivo.

To define the processes required priming the PGCs for motility, we studied the function of the maternally-provided germ-plasm component dead end (dnd) gene. The Dnd protein contains an RNA binding domain and is specifically expressed in PGCs, where it is localized to the perinuclear granules. Knockdown of dead end results in severe defects in cell behavior (e.g. the acquisition of motility), cell fate maintenance and survival. We found that Dnd functions by protecting specific RNAs (e.g. tdrd7 and nanos1) from micro RNA–mediated translational inhibition and RNA degradation in the germ cells, a process by which the same RNAs are inhibited in somatic cells. To determine the molecular basis for Dnd function we have identified proteins that interact with Dnd as well as RNAs that are potentially regulated by the Dnd complex.

The identification and functional analysis of such RNAs that are involved in controlling cell shape and motility will be described.

Website
http://zmbe.uni-muenster.de/institutes/izb/izbmain.htm
Session VI
Mechanobiology of Development and Tissue Morphogenesis
The Subcellular Mechanics of
Tissue Morphogenesis

**Thomas Lecuit**

Developmental Biology Institute of Marseilles–Luminy (IBDML), CNRS & Aix-Marseille University
Campus de Luminy, Marseille, France

Tissues exhibit a remarkable dual property of robustness and plasticity. This relies on unique mechanical properties of the cell cortex and on adhesive interactions between cells. Our group seeks to understand the fundamental molecular mechanisms responsible for this property. This is essential to understand morphogenesis of developing embryos and organs, and is severely affected in a number of diseases. To that end we develop a range of approaches, from the genetic and pharmacological perturbations of molecular components, the quantitative imaging of proteins using a variety of photonic methods, probing of the physical properties of cells within intact tissues, and predictive computational modelling of morphogenesis at different scales (molecular to tissue scales) with two collaborators (PF. Lenne and Ed. Munro).

I will present our current research characterizing how adhesion and cortical tension regulate the dynamic remodelling of cell contacts in the primary epithelium of Drosophila embryos. I will first focus on the regulation of polarized tensile activity driving cell shape changes during cell intercalation. I will also address how E-cadherin-actin interactions control force transmission at cell interfaces.

Website
The Influence of Mechanics and Chemistry on Spatially Patterning Cell Behaviour and Tissue Dynamics: Insights from Drosophila Dorsal Closure

Maithreyi Narasimha

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The amnioserosa, an active participant during Drosophila dorsal closure, exhibits a rich heterogeneity of cell behaviours that include stochastic and collective behaviours to ensure its contraction. We have sought to understand the cellular, molecular and physical bases of individual behaviours and their coordination that ensures the stereotypical dynamics of this tissue. We discuss results from a combination of approaches including targeted genetic and laser induced perturbations, cell biology, 4D live confocal microscopy and quantitative morphological analysis that have provided insights into the origin and nature of cues that pattern cell delamination. Collectively, they uncover local and global, autonomous and non autonomous, mechanical and chemical, genetically hardwired and emergent influences on patterning cell delamination and establish a critical role for cell adhesion in the polarisation of active stresses generated by the cytoskeletal elements. Our findings pave the way for simulations and theoretical frameworks that capture and describe the spatial patterns of cell behaviour we observe.

Website
http://www.tifr.res.in/~dbs/faculty/M_Narasimha.html
Regulation of Dorsal Closure in Drosophila Embryogenesis

JUAN RIESGO-ESCOVAR

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We have isolated and characterized mutations in two loci required for embryonic dorsal closure in the fruit fly Drosophila melanogaster: piragua (prg) and chem. Embryonic dorsal closure is a post-mitotic process whereby two epithelial sheets change shape and elongate to cover the dorsal aspect of a developing embryo. The epithelial sheets that change shape at both sides of the embryo are known as the lateral ectodermal cells. This process is orchestrated by the dorsalmost row of cells at each side of the embryo, the so-called leading edge cells, and depends upon signaling by the JNK (Jun N Terminal kinase) and dpp (decapentaplegic; a transforming growth factor homolog) pathways, amongst others. It is known that both an transcellular actin cable and tubulin polymerization at the leading edge cells, and ‘pulsating’ amnioserosa cells, an extra embryonic epithelia to be covered by the elongating epidermal cells, contribute to the dynamics of dorsal closure. Defects in this process leave dorsal portions of the embryo not covered with the lateral epithelial cells, and, as a consequence, a dorsal open or dorsal hole phenotype, and are generally embryonic lethal. Mutations in piragua have defects in dorsal closure, in cases leaving a small dorsal hole in the cuticle; in other instances embryos close dorsally, but nevertheless die. piragua codes for a zinc-finger containing transcription factor, and mutant embryos have slower closure dynamics, often leading to abnormal closure and ‘zippering’ of the embryos. In contrast, chem codes for a E3 ubiquitin ligase, and interacts genetically with cytoskeletal and signaling proteins required for cell polarity in elongating epidermal cells. There are strong genetic interactions between chem and yurt and other structural proteins. Mutations in chem lead to loss of cell polarity and mislocalization of cell junction proteins.

Website
Mechanotransduction in Development, Conservation Across Species and Evolutionary Incidences

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Biochemical patterning and morphogenetic movements coordinate the design of embryonic development. The molecular processes through which differentiation patterning closely controls the development of morphogenetic movements are today intensively studied. Recent experimental evidence demonstrates that mechanical cues generated by morphogenesis activate mechano-transduction pathways, which conversely regulate the cytoskeleton rearrangement, cell proliferation, tissue differentiation and acto-myosin dependent active morphogenesis of embryonic development (Farge, 2011). Specifically, mechanical activation of the Fog dependent apical stabilisation of Myosin-II rescues mesoderm invagination of early Drosophila embryos mutant of Snail lacking invagination (Pouille et al., 2009). We propose an in silico model that quantitatively phenocopies the transition of collective constriction of mesoderm cell apexes leading to mesoderm invagination in response to the activation of the Fog mechano-transduction pathway to Snail dependent pulsating cells mechanical deformation (Driquez and Bouclet et al). We also show b-catenin dependent mechanical induction of meso-endoderm differentiation in zebrafish early embryos, that parallels the Armadillo/b-catenin dependent mechanical induction of the meso-endoderm gene Twist in early Drosophila embryos (Brunet et al). We describe how the conservation across species of associated mechano-sensitive pathways in adult tissues opens new perspectives on mechano-transduction involvement in evolution (Farge, 2011).

References

Website
Muscle-induced Mechanical Loads Regulate Key Aspects of Skeletogenesis

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The involvement of embryonic movement and muscle contraction in skeletogenesis has long been recognized. Yet, the mechanisms that underlie the role of muscle-induced mechanical load in processes such as joint formation and bone shaping have remained to be elucidated. Synovial joints develop from a pool of progenitor cells that differentiate into various cell types constituting the mature joint. Using several murine models that lack either limb musculature or its contraction, we show that contracting muscles are fundamental in maintaining joint progenitors committed to their fate. In the absence of muscle contraction, the differentiation sequence was disrupted, as joint progenitors assumed a chondrogenic fate. This resulted in impaired joint cavitation and morphogenesis. We then show that contraction-dependent activation of β-catenin, a key modulator of joint formation, is the molecular mechanism that mediates this effect. Our findings link between cell fate determination of organ progenitors and embryonic movement, two processes shown to be essential for organogenesis. In another work, we study the role of intrauterine muscle-induced mechanical loads in determining the complex and unique 3D morphology of developing bones. Analysis revealed that developing mouse bones are subjected to significant and progressively increasing mechanical challenges. Using daily micro-CT scans of appendicular long bones, we monitored the course of structural and mineral changes during development. We identify a developmental program we name preferential bone growth, which determines the specific circumferential shape of each bone by employing asymmetric mineral deposition and transient cortical thickening. Computer models demonstrate that the resulting bone structure has optimal load-bearing capacity. Finally, we use muscular dysgenesis (mdg) mice to show that in the absence of muscle contractions, the stereotypical circumferential outline of each bone is lost, leading to the development of mechanically inferior bones. This study identifies muscle force regulation of bone preferential growth as a common module that shapes the distinctive circumferential outline of each long bone and, consequently, optimizes its load bearing capacity during development.

Website
http://www.weizmann.ac.il/molgen/members/zelzer/index.html
Reconstructing Neural Development

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In embryonic development of vertebrates and higher invertebrates, a single cell is transformed into a fully functional organism comprising tens of thousands of cells, which are arranged in tissues and organs able to perform the most spectacular tasks. A key component that stands out in terms of size, complexity and function is the animal’s central nervous system (CNS). However, very little is known about the developmental dynamics of this complex system, since the technology to comprehensively visualize and computationally analyze in vivo cell behavior in the CNS is lacking. The overall objective of our research is to gain such quantitative experimental access, to determine the fundamental properties of CNS architecture and to attain a system-level understanding of CNS development and function.

Our experimental assays employ light sheet-based fluorescence microscopy, an emerging imaging technology that achieves an exceptionally high imaging speed and excellent signal-to-noise ratio, while minimizing light exposure of the specimen. This unique combination makes light sheet microscopy an indispensable tool for the long-term in vivo imaging of entire developing organisms.

We are designing advanced implementations of scanned light sheet-based fluorescence microscopy to study the early development of fruit flies and zebrafish. We are furthermore devising new strategies for automated large-scale image processing and optimizing specimen culturing techniques and transgenic reporter lines. These tools allow us to perform whole-organism functional imaging and to quantitatively analyze developmental lineages and their interrelationships in the entire animal. Our goal is to take advantage of these high-resolution data to attain a system-level understanding of cell fate decisions and how they establish the dynamic architecture of neural tissues. In the long-term perspective, we will use this information for the establishment and validation of a morphogenetic model of CNS development.

We envision that our quantitative approach to the reconstruction of large neuronal systems dynamics will provide critical insights into the properties of complex circuits and complement ongoing large-scale electron microscopy analyses of static neuronal network architecture.

Website
http://www.hhmi.org/research/fellows/keller_bio.html
Mechanical Forces Driving Zebrafish Epiboly

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The molecular and cellular mechanisms by which tissues take shape are fundamental to many biological processes. While the genetic pathways controlling tissue morphogenesis have been intensively analyzed, its mechanical principles are poorly understood. An excellent assay system to study the biophysical basis of tissue morphogenesis is zebrafish gastrulation, where within a few hours major morphogenetic changes result in the formation of germ layers and the establishment of the body axis.

To obtain insight into the biophysical basis of tissue morphogenesis during gastrulation, we study enveloping cell layer (EVL) epiboly, the spreading of a squamous epithelium over the yolk cell. A circumferential actomyosin band within the yolk syncytial layer (YSL) has been proposed to act as a purse string pulling on the EVL margin. However, direct evidence supporting this hypothesis has been missing. Using laser ablation to measure tension within the actomyosin band, we find an anisotropic tension distribution with highest tension parallel to the EVL margin. Notably, there is also tension perpendicular to the EVL margin, indicating that the actomyosin band is not free to constrict in this direction. To understand how anisotropic tension within the actomyosin band controls EVL epiboly, we have started with a hydrodynamic description of this process, modeling the actomyosin cortex as an active, viscoelastic gel. Initial quantifications of cortical flows within the EVL and YSL are consistent with predictions from our theory, supporting the general plausibility of our theoretical approach.

Based upon initial results, we propose a new mechanism for the actomyosin band in EVL epiboly: in addition to function as a geometry-dependent purse string, it exerts a friction-based pulling force. Currently, we are analyzing the generation of this force and its contribution to epiboly.

Website
http://ist.ac.at/research/research-groups/heisenberg-group/
In Zebrafish, maternal dorsal determinants are vegetally localized in the egg and transported after fertilization. Transport of dorsal factors is dependent on microtubules. However, microtubule dynamics at these early stages of development has not been examined. Using transgenic reporters, we analyzed microtubule dynamics by live imaging in eggs & embryos, and identify three microtubule populations at the vegetal cortex:

1) Perpendicular arrays, a novel population emanating from the vegetal pole & directed towards the blastoderm
2) Parallel bundles, that move towards one side of the cortex, and
3) Non-directional meshwork

Perpendicular and parallel arrays are detected only at the vegetal cortex, and are extremely transient, observed only before the 1st cell division. Parallel bundles, covering a region ~250 µm in diameter, move asymmetrically to one side of the vegetal cortex. Yolk granules in the vegetal cortex are displaced by ~15° together with the parallel bundles, within 30 minutes post-fertilization. Displacement of the cortex likely contributes to transport of determinants to one side of the embryo. The non-directional microtubule meshwork is more persistent and abundant than the parallel and perpendicular arrays, but is also dynamic. This meshwork appears after the parallel arrays are disassembled. Organization of the microtubule meshwork changes periodically with a timing similar to that of the cell cycle. We observe waves of remodeling microtubules traversing from the animal pole to the vegetal pole. Interestingly, interfering Ca²⁺ signaling in early embryos affects microtubule dynamics and the transition from parallel arrays to the non-directional meshwork is delayed. Our results show that microtubules are continually remodeled during early zebrafish embryogenesis, and provide insights into cytoskeletal dynamics in the control of early patterning.

Website
http://www.tll.org.sg/group-leaders/karuna-sampath
Poster Presentation Abstracts
Nuclear Reorganization and its Coupling to Gene Function During T-Cell Activation

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Naive T-cells in physiology emerge from the thymus into circulation and are subjected to shear stress arising due to blood flow and transmigration. However in this mechano-chemical process, the spatio-temporal alterations in nuclear organization and its coupling to T-cell activation gene markers are still unclear. In this study we reveal an intriguing heterogeneity in chromatin assembly in naive T cell population; marked by central and peripheral condensed DNA in the nucleus. Upon in vitro activation of T-cell receptors, using surrogate antigens anti CD3-anti CD28 coated magnetic beads, the heterogeneity in chromatin assembly is drastically reduced with centrally positioned condensed DNA and increase in nuclear volume by day-two cultures. Time-lapse imaging of early T-cell activation within the first hour captures the early steps of nuclear reorganization concomitant with cytoskeletal polarization with respect to T-cell receptors. Interestingly application of mechanical force on anti CD3-anti CD28 magnetic beads during T-cell receptor engagement resulted in a larger fraction of T-cells expressing CD69 gene activation markers. An inhibitor screen of CD69 pathway revealed actin-mediated cytoskeletal links and Lck-Fyn, Erk and NFkB in the mechanosignal transduction pathway. Taken together these results provide an essential role for nuclear reorganization in regulating gene expression during both early and late T-cell activation response.
Probing the Functional Integration of Mechanosignals to Chromatin Organization in Living Cells

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Cells sense physical and chemical cues from their microenvironment and selectively transmit these cues to the nucleus – resulting in altered gene expression. However the underlying mechanisms are still unclear. In this work, we provide evidence for transduction of physical forces between the plasma membrane and the chromatin organization through the actin cytoskeleton. For this controlled forces are applied using an electromagnet on cell membranes adhered with magnetic beads. The effect of force and its transduction to the nucleus is visualized using high-resolution fluorescence anisotropy imaging of chromatin assembly within single living cells. Our studies evidence physical links anchoring cytoskeleton and the nucleus. Force induced disruption of these links relaxes the prestressed organization of the cell nucleus in a reversible manner. In addition cellular networks are shown to be correlated with 3D organization of chromosomes and their gene expression patterns. Taken together our results evidence an architectural coupling between physico-chemical networks and spatial organization of the chromosomes within the nucleus facilitating mechanotransduction.
Involvement of Formins in the Organization and Dynamics of Golgi Apparatus

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Golgi apparatus, a central element in the intracellular trafficking, consists of ribbon-like structures made of membrane stacks. Here we demonstrate that two potent promoters of actin polymerization, mDia1 and DAAM1 formins, play a role in the maintenance of Golgi integrity. In our recent paper (Zilberman et al, Mol. Biol. Cell, 2011, 22:2900-2911) we showed that Rho-mediated mDia1 activation resulted in pronounced fragmentation of the Golgi into ministacks. Moreover, the formation of Rab6-positive transport vesicles derived from the Golgi complex was enhanced upon activation of the Rho-mDia1 pathway. Here we show that overexpression of active mDia1 resulted in the decrease of speed and directionality of Rab6-positive cargos. Inhibition of actin polymerization by latrunculin, cytochalasin, or SMIFH2 (small molecule inhibitor of formin) restored the Rab6 vesicles movement. Another member of formin family DAAM1 has been implicated in Golgi positioning and directed cell migration (Ang et al, PLoS One, 2010, 5, pii:e13064). Here we show that DAAM1 is localized to the Golgi and has a potential role in maintenance of the Golgi structure. Full length DAAM1 and a GFP fusion protein containing 1-233 amino acids were shown to localize to the Golgi membranes. Overexpression of active DAAM1 in HeLa cells resulted in transition from compact ribbon Golgi structure to smaller punctate structures distributed around the nuclei. Nocodazole washout experiment performed in cells expressing active DAAM1 indicated that smaller Golgi elements fail to fuse into a larger Golgi ribbon, indicating to a role for DAAM1 in the regulation of Golgi membrane fusion.
Stochastic Failure Mechanics of F-Actin Networks: A Computational Approach

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Fibrous networks are subscale motifs ubiquitous in a variety of natural and artificial systems at protein length-scales including tissues, cells, paper, synthetic polymers, and so on. In biological cells, fibrous networks at different length-scales, such as actin, intermediate filaments and microtubules mediate the mechanical response. Unlike engineering materials, intriguing variability is seen in the mechanical response of such structures which is attributed to the network architecture, dynamic rearrangements and sensitivity to a host of chemical and mechanical signals. Understanding the behavior of cells is important in view of the wide range of promising applications in the field of medicine and biomimetics.

The focus of this work is modeling the failure mechanics of fibrous structures mimicking F-actin networks. The cell cortex has a fluid filled structure reinforced by filaments and F-actin crosslinked by a variety of actin binding proteins dominates the cell response. The mechanical response of the F-actin networks is highly nonlinear showing distinct regimes- initial flimsy response, strain hardening followed by softening and collapse. This can be attributed to the corresponding bending dominated reorientation of F-actin filaments to the loading direction, stretching of the filaments, force-dependent crosslink scission and colossal scission at higher forces. We model the nonlinear response of the F-actin architecture networks in a 2D computational framework using finite element analysis. Filaments are modeled as beam elements and crosslinks as elastic springs. The crosslinks are endowed with an ability to dissociate in a stochastic manner, which is modeled using the Kinetic Monte Carlo procedure. This induces a rate-dependent crosslink scission that provides overall rate-dependent stiffness-strain and failure characteristics. We discuss the variability in the rate-dependent response arising from the topological randomness and stochastic effects of crosslink scission as a function of network parameters, stiffening and failure.
Molecular Characterisation of a Novel Actin Network at the Apical Side of Epithelial Cells

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The actin cytoskeleton is responsible for the generation and maintenance of cell shape and dynamic processes like cell motility. Animal cells perform these tasks using a variety of discrete actin structures. Previous work in our group identified a novel actin network located at the apical side of epithelial cells. The network is organized into ≤ 2 μm long, wavy or bent actin-filament bundles, which are subjected to characteristic movements and were subsequently termed “actin worms”. The occurrence of actin worms is restricted to non-confluent epithelial cells, suggesting an involvement in cell motility. The molecular composition of the network, except for actin itself, remains to be elucidated. To address this question we performed a candidate approach using GFP- and RFP-fusions of known actin-binding and -regulating proteins together with live cell microscopy to identify additional molecular components localizing to the actin-worm network. Our results show that, on the molecular level, the network resembles microvilli from the brush border of intestinal epithelial cells. However, the dynamic properties of the actin-worm network are clearly distinct from the rather static behavior of microvilli. Future studies using gene specific knockdown of actin worm components will help to gain a functional understanding of the network.
Regulating Motor Force in Skeletal Muscle Contraction

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In various active biological contractile processes, an interesting common observation is that multiple motors can perform coordinated and synchronous actions while individual myosin motors appear to randomly attach to and detach from actin filaments. Recent experiment has demonstrated that, during skeletal muscle shortening at a wide range of velocities, individual myosin motors maintain a force of about 6 pN during a working stroke. Here we develop a molecular model within a coupled stochastic-elastic theoretical framework. The model reveals that the unique force-stretch relation of myosin motor and the stochastic behavior of actin-myosin binding cause the average number of working motors to increase in linear proportion to the filament load, so that the force on each working motor is regulated at 6 pN, in excellent agreement with experiment. The present work suggests that it might be a general principle in biology to use catch bonds together with a force-stretch relation similar to that of myosin motors to regulate force homeostasis in many biological processes.
Integrin Adhesion Drives the Emergent Polarization of Active Cytoskeletal Stresses to Pattern Cell Delamination

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Cells in epithelia respond to and integrate a variety of biochemical and mechanical cues to generate stereotypic patterns during development and homeostasis. We investigate how cells of the amnio-serosa, an active participant of Drosophila dorsal closure, respond to local mechanical stimulus. We show that a subcellular laser perturbation (that ensures plasma membrane integrity) triggers cell delamination/extrusion. An initial phase of cell expansion precedes extrusion enabling us to estimate cellular prestress (tension). We characterize the spatial reorganization of the cytoskeleton and the temporal hierarchy of their deployment in the perturbed cell and its nearest neighbours during cell delamination. We find an emergent polarization of actin (lamellipodial protrusions and cortical enrichment), myosin (streaming movement of medial myosin and cortical enrichment) and microtubules (polarized rearrangement) at the interface of the perturbed cell and its nearest neighbours in response to the perturbation. We identify novel roles for microtubules- in the streaming movement of myosin and timely completion of delamination in response to the subcellular perturbation. We demonstrate that the reorganization of actin and myosin is dependent on integrin adhesion and precede changes associated with microtubules. Our results indicate that cell delamination is patterned locally through differential contributions of adhesion, cytoskeleton and local mechanical cues.
Vinculin - A Transducer of Mechanical Force Regulating the Recruitment and Release of Focal Adhesion Proteins

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Although initially described as a mechanical link between the cell and surrounding extracellular matrix, it is now clear that focal adhesions (FAs) act as integrators of physical cues and cellular responses. However, very little is known about the conversion of a mechanical signal into a biochemical response. Here, using cycling stretching stimulation, atomic force spectroscopy, and live imaging techniques, we show that vinculin is an important mechanotransducer of FAs and this function relies on actomyosin mediated tension and attachment to talin. Under intracellular tension, vinculin switches from globular to extended conformation and displays additional sites for binding partners. The use of various vinculin mutants demonstrates that vinculin activity regulates the turnover of FAs by influencing integrin activation and controls the binding of many FA components. Moreover, we confirm that preventing vinculin from folding back to its globular conformation leads to slower migration speed and a defect in the establishment of cellular polarity. Altogether, this work emphasizes the role of vinculin as a transducer of mechanical stimulation into intracellular signalling cues.
Focal Adhesion Kinase (FAK) Alters Cell Morphology and Migration on Microstructured Polymer Substrates

Maruxa Estévez, Elena Martínez, Stephen J. Yarwood, Matthew Dalby, Josep Samitier

Microstructured substrates have emerged in recent years as a useful tool to elucidate the influence of the mechanical environment that cells encounter in vivo during migration. During migration, the FAK is known to be involved in focal adhesion turnover and GTPase activation at the leading edge. In this work, our objective was to determine the importance of FAK in cell morphology and migration responses to microstructured substrates. For this purpose, the motility of mouse embryonic fibroblasts (MEFs) and FAK knocked-out MEFs (FAK-/-) was monitored on polymer microstructured substrates by time lapse microscopy and their morphology assessed by immunostaining. Results showed that the motility of fibroblasts on micropost surfaces was significantly higher than on flat controls as a consequence of the cytoskeleton mechanical instability induced by the surface features. However, the FAK-null cells did not present a differential migratory behaviour on the microstructured posts, which indicates that FAK is essential not only for cell migration but also for cell response to the mechanical stimuli generated by the microstructures.
Regulation of Force Generation by p53 and NF-κB: Mechanical Implication of Cancer Progression


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Tumor suppressor p53 is reported to suppress activity of NF-κB pathway. Consistently, NF-κB is constitutively activated in p53-deficient cells as well as numerous malignant cells. However, it remains unclear how NF-κB regulates tumor progression such as metastasis. We first examined whether NF-κB was involved in the regulation of force generation that is associated with cell migration, hence invasiveness and metastasis. Using traction force microscopy, we found that the traction forces generated by p53-deficient mouse embryonic fibroblast (p53⁻/⁻ MEFs) were significantly decreased upon NF-κB knockdown or introduction of p53. Since focal adhesion proteins such as integrins, FAK and p130Cas are involved in the transmission of traction forces, we tested whether NF-κB modulated the functions of these molecules. Knockdown of NF-κB expression affected integrin κ3 phosphorylation, while it did not lead to significant alteration of the expression/phosphorylation of p130Cas and FAK. In addition, NF-κB knockdown in p53⁻/⁻ MEFs resulted in a decrease of integrin κ3 at focal adhesion sites. Furthermore, traction forces generated by p53⁻/⁻ MEFs were decreased when treated with the integrin κ3 blocking antibody. These results suggest that NF-κB up-regulates traction force generation through modification of integrin κ3, which is related to metastasis.
BNIP-2 Promotes BPGAP1 Inactivation of Rho and Changes in Morphology via Heterophilic Interaction of Their BCH Domains

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Amongst many ways protein functions are executed and regulated, protein-protein interactions between identical or homologous domains represent a key and common mechanism. The BCH (BNIP-2 and Cdc42GAP Homology) domain represents an emergent class of protein modules with such property. Interestingly, it has the ability to interact with and regulate the activities of Rho small GTPases and their immediate regulators, GEFs or GAPs. We recently reported that BPGAP1, a BCH domain-containing homolog of p50RhoGAP, induces cell migration and Ras-ERK activation by interacting with diverse partners. However, little is known about how its function(s) is modulated by other BCH domain-containing proteins. Here we show that BPGAP1 forms a physiologic complex with BNIP-2 via heterophilic interaction between their BCH domains but also unsurprisingly between BNIP-2 BCH domain and the RhoGAP domain. Interestingly, a consensus RhoA-binding motif exists within the BCH domains of BNIP-2 and BPGAP1 and screening of various Rho GTPases revealed that both proteins targeted preferentially Rho A and Rho C, but not RhoB, raising the possibility that their interaction could modulate specific Rho signaling. GTPases assays and bioimaging studies revealed that BNIP-2, BPGAP1 and RhoA colocalized at the cell edges, and they synergistically enhanced the RhoGAP activity of BPGAP1 towards RhoA, leading to reduction in stress fibers and cell proliferation, and increase in GAP-dependent cell rounding. This process could involve displacing the autoinhibition of BPGAP1 or recruitment of RhoA by BNIP-2 towards BPGAP1. BNIP-2 therefore presents a novel regulator of BPGAP1 at least in cell morphogenesis and proliferation, and their implications on single and multicellular cell migration and cellular transformation will be discussed.
Cross-Species Genome-Wide Analyses Identify BNIP-2 And Cdc42gap Homology (BCH) Domain as a Distinct Functional Subclass of the CRAL-Trio/Sec14 Superfamily

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Although functions of small GTPases and their control by guanine nucleotide exchange factors (GEFs) and GTPase-activating Proteins (GAPs) are well established, it remains unclear whether GTPases, GEFs and GAPs themselves are regulated by other cellular factors. We have recently identified BNIP-2 and Cdc42GAP Homology (BCH) domain as a novel regulatory scaffold that targets Rho small GTPases, RhoGAPs and RhoGEFs to affect cell signaling and dynamics under different conditions. The BCH domain shares low sequence homology to the CRAL_TRIO domain of Sec14 superfamily, which binds to diverse, small and hydrophobic ligands. Using extensive phylogenetic clustering, we report ~200 BCH domain-containing proteins from diverse organisms and establish its clear distinction from the CRAL_TRIO domain. We propose that BCH acts a ‘novel subclass’ of CRAL_TRIO domain recognized with its hallmark sequence motif R(R/K)h(R/K)(R/K)L(R/K)xhhhhHPs (where ‘h’ is large hydrophobic and ‘s’ is small weekly polar residue). We further classified BCH into three groups and proposed features unique to each group, including how BCH domain could have evolved through intron insertions and domain swapping. Understanding the point of divergence and unique features between BCH and CRAL-TRIO should therefore help us understand the emergent role and plasticity of BCH domain in regulating small GTPases signaling and possibly the lipids.
Dynamics of Cell-Cell Junction Reinforcement Probed with High Resolution Microscopy

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We present a new experimental approach to study the dynamics of formation of adherens junctions. Cells are individually added to 3D microwells to form duplexes or triplexes. The initial contact time is well defined and the 3D microstructure allows us to control the physical and chemical environment surrounding the cells. Using an appropriate geometry, we form cell-cell contacts parallel to the focal plane of the microscope objective. The dynamics of the contact area is then imaged at high spatial and temporal resolution by spinning disc microscopy. Few minutes after cell contact is established, the cortical actin is disrupted within the contact zone and rapidly forms an actin ring at the growing rim of the contact area. The E-cadherins cluster into five to ten foci per square micron that subsequently assemble into a punctuated ring colocalized with the actin ring. We then compare this formation dynamics depending on the preexisting engagement of the cells in integrin based adhesion with the surrounding substrate. Lastly we present a mechanical model accounting for the reinforcement of cell adhesion with time based on the spatial arrangements of actin and cadherins.

A

B

A: Schematic representation of the experimental set-up. B: Time-lapse images of the E-cadherin GFP at the cell cell junction
Cortactin is a Central Organizer of Actin and E-Cadherin at the Zonula Adherens

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Cortactin is an F-actin-binding protein that localizes to the zonula adherens (ZA) in simple epithelial cells. It was first identified as a Src substrate, and its activity is regulated via phosphorylation by Src and other kinases. Cortactin interacts with E-cadherin, and contains multiple domains that bind important actin regulatory proteins including the actin nucleator Arp2/3. Thus, cortactin can potentially act as a scaffolding protein to recruit and organize key players in actin dynamics to the ZA. To study this, we generated lentiviral-infected cell lines in which cortactin was knocked down and reconstituted with wildtype or phospho-deficient cortactin, and examined the effects on ZA integrity. We found that loss of cortactin decreases both steady state F-actin levels and actin nucleation at the ZA in an Arp2/3-dependent and phosphorylation-independent manner. In contrast, decreased E-cadherin levels at the ZA in cortactin knockdown was not fully restored by phospho-deficient cortactin. In an effort to identify molecules responsible for these regulatory effects on the ZA, we are performing a proteomics screen for interacting partners of cortactin. Our results establish the central role of cortactin in actin cytoskeletal regulation at the ZA, and suggest mechanisms that integrate cadherin organization, actin dynamics and kinase signaling at intercellular junctions.
Cadherin Exits the Junction by Switching its Adhesive Bond

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The plasticity of cell–cell adhesive structures is crucial to all normal and pathological morphogenetic processes. The molecular principles of this plasticity remain unknown. Here we study the roles of two dimerization interfaces, the so-called strand-swap and X dimer interfaces of E-cadherin, in the dynamic remodeling of adherens junctions using photoactivation, calcium switch, and coimmunoprecipitation assays. We show that the targeted inactivation of the X dimer interface blocks the turnover of catenin-uncoupled cadherin mutants in the junctions of A-431 cells. In contrast, the junctions formed by strand-swap dimer interface mutants exhibit high instability. Collectively, our data demonstrate that the strand-swap interaction is a principal cadherin adhesive bond that keeps cells in firm contact. However, to leave the adherens junction, cadherin reconfigures its adhesive bond from the strand swap to the X dimer type. Such a structural transition, controlled by intercellular traction forces or by lateral cadherin alignment, may be the key event regulating adherens junction dynamics.
Modulating Nuclear Organization Alters Segmental Gene Expression Patterns in Drosophila Embryos

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The nucleus is maintained in a prestressed state within eukaryotic cells, stabilized mechanically by chromatin structure and other nuclear components on its inside, and cytoskeletal components on its outside. Recent evidence suggests that the inter-cellular connections mediated by various adhesion proteins contribute to defining nuclear morphology and that transcription programs are regulated by the 3D organization of the cell nucleus. However the emergence of prestressed nuclear organization and its coupling gene expression is poorly understood. In this work, we study the changes in nuclear morphology during both stem-cell differentiation and in developing Drosophila embryos. Time-lapse imaging experiments reveal spatio-temporal changes in nuclear morphology and its stiffness during this process. Further we use a point perturbation by tissue level laser ablation and sheet perturbation by application of force using magnetic trap to alter cellular morphogenetic movements and probe its impact on nuclear morphology and gene expression in developing Drosophila embryo. Physical perturbations during blastoderm stage resulted in localized alterations in nuclear morphology and cellular movement. Further application of local force resulted in non muscle myosin-II redistribution in the germ band layer. Finally these perturbations result in altered segmental gene (en-grailed) expression patterns. Our results highlight the functional importance of prestressed nuclear morphology during cellular differentiation and development.
Spatio-Temporal Organization of Transcription Factories within Living Cells

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Recent studies have shown that transcriptional activity in the nucleus is organized in distinct sites called transcription compartments or factories (TFs), where genes either loop out or are co-clustered to form active chromatin hubs. In addition, our studies are beginning to reveal that gene-active chromosomes share physical proximity within the 3D architecture of the cell nucleus. However the spatio-temporal organization of TFs and its functional implications are unclear. Using high-resolution live-cell fluorescence imaging and spectroscopy, we analyze the dynamic organization of TFs. For this we labelled TFs using fluorescent UTPs which co-localize with active RNA Pol-II antibody in a transcription dependent manner. Dual color labelling methods revealed that TFs are specialized foci. Further, the local chromatin structure, mapped using fluorescence anisotropy measurements, in vicinity of TFs showed a transcription dependent compaction state. Interestingly TFs exhibited a dynamic behaviour with runs, pauses and steps. This dynamic organization of TFs was dependent on ATP, lamin B1, histone acetylation levels and cytoplasmic to nuclear anchorage. Importantly during runs, TFs are mobile within the inter-chromosome territories. The spatio-temporal organization of TFs that we observe may provide possible mechanisms to alter gene expression programs upon integration of physico-chemical signals to the nucleus.
Myosin IIB-Dependent Junctional Tension is Regulated by an RPTP-α/Src Signaling Module

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Cell-cell adhesion sites serve to sense the mechano-biological properties of cells and their environment, where cells generate and respond to forces originated by their neighbors and also activate signaling pathways. However, little is known about the molecules involved in force generation and the signaling pathways that control it. By analyzing cell-cell contacts movements, we found they are continuously contracting and relaxing, a process necessary for cell-contact remodeling and collective cell movement. Drug treatment and RNAi experiments identified myosin IIB as a key determinant of cell-cell contact contractibility or junctional tension, a conclusion that was further confirmed by nano-dissection. In order to characterize the regulators of myosin IIB activity at the cell-cell junctions, we found that Src inhibition causes loss of Myosin IIB but not IIA from junctions, reduces junctional tension and alters the linear morphology of cell contacts. We also found that junctional Src activity is controlled by RPTPα. Knockdown of RPTPα causes a reduction of Src activity and junctional tension. These results imply a key role of RPTPα/Src signaling module in Myosin IIB regulation. Our results suggest this pathway is important for high order cellular organization through the mechanical coupling between neighboring cells at cell-cell contacts.
Myosin 1s Move Directionally on Active Lamellipodial Surface

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Because the actin network in active lamellipodia is continuously assembling at the edge, moving inward at speeds up to 200 nm/s and disassembling, there is a question about how actin binding proteins and edge receptors are transported to the leading edge. There has been some indirect evidence of an active transport system but the basis of that transport is unknown. We show here that several myosin 1 isoforms (1G/1B/1C/1F) rapidly move to the leading edges of lamellipodia at different rates up to 5 μm/s. Photoactivation of PAmCherry-myosin 1G shows rapid outward movement on the upper surfaces of lamellipodia. Movement depends upon PH domains for lipid binding and upon ATPase activity. Surprisingly, at membranes bound to fibronectin-coated surfaces (ventral surface of lamellipodia), activated myosin 1G particles move in all directions; although at rapid rates (average velocity 3.4 ± 1.23 μm/s). Thus, it appears that myosin 1s actively move on dorsal actin to the leading edge and are not carried by fluid flow or some other biophysical mechanism. We suggest that myosin 1s transport actin, actin-binding proteins and some membrane receptors to the leading edges of lamellipodia to enable treadmilling. Further, myosin 1s can concentrate proteins at sites of actin polymerization.
Actin Cytoskeleton Signaling Regulates Mitotic Spindle Orientation

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The actin cytoskeleton is a dynamic network that gives eukaryotic cells their characteristic shape. However, it undergoes extensive reorganization and remodeling during mitosis. Adherent cells, which adopt ‘spread out’ morphologies during interphase, exhibit a dramatic loss of stress fibres upon entering mitosis and become rounded. The cortical actin network becomes more prominent during this time, resulting in an increase in cortical rigidity. Upon completion of cell division, the daughter cells re-establish attachment to extracellular matrix and once again attain the spread-out morphology. While the regulatory connections between the actin cytoskeleton and the early mitotic events are apparent, the mechanisms that govern these links are not well understood. In this study, we investigated the functional links between the actin cytoskeleton and the cell division cycle at early stages of mitosis. We found that perturbation of the cortical actin by the inhibition of RhoA and ROCK led to aberrant astral microtubule phenotype and mis-orientation of the mitotic spindle. We have also uncovered the signaling pathway involved and found LIMK2 playing a critical role in organizing the astral microtubules.
Mechanosensing in a Novel Actin Network at the Apical Side of Epithelial Cells

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The cytoskeleton plays a central role in morphogenesis by generating, sensing and transmitting physical forces. Actin filaments are mostly arranged close to the plasma membrane, forming distinct higher order assemblies such as lamellipodia, filopodia or stress fibers. We have identified highly dynamic actin structures at the apical side of non-polarized epithelial cells. Remarkably, movement and reorganization of these “actin worms” is driven by an underlying non muscle myosin II network covering the whole apical cell surface. Through application of shear flow, we demonstrate that the actin worm network is highly sensitive to external mechanical perturbations. After only few minutes of applying constant shear stress, actin worms completely disassemble, while at the same time high number of actin bundles assemble around the nucleus. When kept under constant shear stress over long timescales, actin worms slowly reassemble and again form a dense apical network. The remarkably instantaneous rearrangement of cortical and perinuclear actin structures will be used as an assay to study cellular mechanosensing i.e. the mechanisms for propagation of mechanical signals within cells.
Eukaryotic cells modulate their gene expression profile and differentiation in response to physical and chemical cues in the micro-environment. The effect of the stiffness of the substrate on cell spreading and differentiation has been of interest recently. The exact mechanism by which the cells sense the stiffness of the substrate has not been very well understood. Studies with micropillar arrays suggest the cells are rather insensitive to the stiffness and displace the pillars for ~130 nm irrespective of the stiffness and might be integrating the force required. These studies used pillars of a few micron squared surface area and hence the cells might be integrating the force/displacement between multiple contacts on the same pillar. My project aims at restricting the contact area available to the cell to one focal contact per pillar and to see the response of cells on these pillars.
Specificity and Functional Cooperation of the Fibronectin-Binding Integrins αvβ3 and α5β1 in Focal Adhesion-Mediated Signal Transduction

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The 24 α/β-heterodimers of the integrin family mediate cell adhesion by connecting the extracellular matrix (ECM) to the actin cytoskeleton. This linkage results in the formation of multi-protein assemblies called focal adhesions (FA) where mechanical forces, cytoskeletal organization and biochemical signals intersect and a plethora of cellular functions including cell migration, proliferation, differentiation and survival are modulated. It has been shown that FA-mediated signaling events downstream of two integrin heterodimers, even when bound to the same ligand (such as α5β1 and αvβ3 bound to fibronectin) can be profoundly different. The expression of multiple integrins on a given cell type renders the precise analysis of such signaling specificity extremely difficult. Therefore, it is mandatory to reduce the complexity of the network to gain insight into the specialized functions of individual integrins. Using genetically modified mice we generated integrin-negative fibroblasts, which were reconstituted with either β1 cDNA, αv cDNA or both. The reconstituted cells were used as a unique model system to study signaling specificity and crosstalk of the two major fibronectin (FN) binding integrins α5β1 and αvβ3. Using various methods including confocal microscopy of cells on FN coated-micropatterns we characterized the phenotype of α5β1 versus αvβ3 expressing cells and uncovered specific signaling intermediates and functional synergies downstream of the two integrin heterodimers. The data will be presented and discussed at the 5th Mechanobiology Conference at the National University of Singapore.
Combinatorial Effects of Mechanical Loading and RGD Containing Substrates on Cell Functions in Mesenchymal Stem Cells

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Bone regeneration depends on mesenchymal stem cells (MSC) controlled by environmental factors like the extracellular matrix and mechanical forces. Therefore, this study aims at analyzing combinatorial effects of mechanical loading and RGD containing material surfaces on cell functions.

For allowing specific cell adhesion, surfaces were bio-functionalized using linear RGD peptides or fibronectin. To apply mechanical stress, β1-integrins on the apical cell surface were mechanically loaded using paramagnetic microbeads. Mechanically stressed integrins induced e.g. an increased expression of collagen I when cells adhered on RGD peptides or fibronectin but not on non-functionalized surfaces. To uncover possible cellular mechanisms, cell spreading and the structural organization of cytoskeletal components were investigated revealing a faster progress on fibronectin compared to RGD peptides and non-functionalized surfaces. Compared to RGD peptides, the area of focal contacts was significantly decreased on fibronectin one day after cell seeding.

In summary, immobilized RGD peptides were sufficient to promote mechanically induced expression of collagen I even though immobilization of fibronectin allows a faster organization of cell adhesion components. In conclusion, fibronectin and RGD peptide modified surfaces play an important role in tissue engineering strategies.
Substrate, Focal Adhesions, and Actin Filaments: a Mechanical Unit with a Weak Spot for Mechanosensors

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It is the fundamental nature of cells to interact with their environment by chemical and mechanical signals. Signals of the latter type such as strain are currently believed to be sensed by focal adhesions (FAs) and the actin cytoskeleton. However, the underlying mechanism and the location of mechanosensory elements are still largely unknown. Here, we developed a method to apply spatially decaying substrate deformation fields to cells. Displacement fields were recorded simultaneously with live cell microscopy on fluorescent proteins of focal adhesions or actin. These experiments enabled a precise mapping of cellular deformation.
Impacts of BNIP-2 in Regulating RhoA-ROCK-Myosin II During Cell Spreading and Collective Cell Migration

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Cell attachment to extracellular matrix and the spreading of cell membrane are essential steps leading to cell migration. It requires tight control of cytoskeletal rearrangement and contractile forces mediated by Rho small GTPase and myosin II, an actin based motor protein. We recently identified BNIP-2 as a novel regulator of Rho leading to cell protrusions and muscle differentiation. However, its function in regulating cell spreading and multicellular/cell-sheet migration remains unclear. Using bioimaging, force-measurement on micropillars, pharmacological inhibition and biochemical assays, we show that the overexpression of BNIP-2 in MDCK cells exerts greater traction force and impairs cell spreading in substrate dependent manner, and it also retards collective cell migration and HGF-induced cell scattering. Interestingly, BNIP-2 inhibition on cell spreading involves activating myosin II via RhoA and Rho Kinase (ROCK) but the inhibition on collective cell migration involves activating myosin II via a ROCK-independent pathway. These findings reveal a novel role of BNIP-2 in regulating distinct Rho signaling underlying cell spreading and cell migration and could have implications on cell motility during tissue development and cancer metastases.
BPGAP1 Coordinates Rho and Cortactin for Cell Polarity and Cell Invasion Through Mek2 And Pin1

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Rho small GTPases regulate actin and microtubule cytoskeleton networks during cellular morphogenesis, motility, invasion, proliferation and tissue development. Although these processes are also under the influence of Erk, a key kinase component of the Ras/MAPK pathway and cortactin, a cortical actin-binding protein that facilitates actin branching and polymerization, it is unclear where and how the signaling of Rho, Erk and cortactin converge and are co-regulated inside the cells. We recently identified BPGAP1 as a potent multi-domain Rho GTPase-activating protein (GAP) that not only biochemically acting to inhibit RhoA activity via its RhoGAP domain, it also promotes Erk activity and cell motility via its BNIP-2 and Cdc42GAP Homology (BCH) domain and the Proline-rich Region (PRR). This PRR target cortactin and endophilin to promote cell motility and EGF receptor endocytosis, respectively; both involving their SH3 domains specifically recognizing the prolines 184 and 186 of the 182-PRPPLP-189 moiety. Interestingly, 186-PPLP-189, which subtly overlaps with the cortactin-binding site, is also a prime target of the WW domain of peptidyl-prolyl cis/trans isomerase Pin1. Consequently, binding of Pin1 to BPGAP1 enhances its RhoGAP activity but it also suppresses the ability of BPGAP1 in inducing acute Erk activation necessary for cell motility. Furthermore, active Mek2 promotes Pin1 binding to BPGAP1 to suppress BPGAP1-induced acute ERK activation and cell migration (Pan et al., J. Cell Sci, 2010). These results raise the question as to whether Pin1 could also affect the function of BPGAP1 with cortactin to regulate cell motility and whether this effect is linked to Mek2/Erk regulation. Life imaging of BPGAP1 and cortactin show their prominent co-localization on membrane ruffles during cell spreading, enhanced cell motility and invasion, whereas over-expression of BPGAP1 and cortactin alone did not result in such dynamic processes. However, Pin1 abolishes such phenomenon. In addition, over-expression of the Rho-GAP inactive mutant, R232A of BPGAP1 with cortactin or treatment with either the Rho inhibitor (C3 transferase protein) or p160ROCK inhibitor (Y-27632) with cells overexpressing wild-type BPGAP1 and cortactin do not inhibit the extensive lamellipodia protrusion but the contractility and polarity of cells were compromised. Consistent to cell imaging studies, co-immunoprecipitation data show that BPGAP1/cortactin interaction was enhanced in the presence of active Mek2 but suppressed by Pin1. The significance of BPGAP1 in coordinating Rho signaling and cortactin function via the new duet regulator of Mek2 and Pin1 in cancer cell metastasis will be discussed.
PINCH1 is a Tension-Sensitive Regulator of Cell-Matrix and Cell-Cell Adhesions in Mouse Epidermis

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The LIM-only domain only focal adhesion (FA) protein PINCH1 forms a ternary complex with integrin-linked kinase (ILK) and parvin (IPP complex). The IPP complex interacts with the cytoplasmic tail of β integrin subunits and transduces signals that are important for cell adhesion, spreading, migration, proliferation and survival. We show that keratinocyte-restricted deletion of the PINCH1 gene in mice leads to epidermal detachment from the basement membrane (BM), epidermal hyperthickening and progressive hair loss, all of which resemble the phenotype of mice lacking ILK in skin and underscore the tightly linked functions of the IPP complex members. However, PINCH1-deficient keratinocytes also display cell-cell adhesion defects which are not observed in epidermis upon ILK loss, suggesting an IPP complex-independent role for PINCH1 in skin. Interestingly, PINCH1 localization to cell-cell adhesions is a force-dependent process which mechanistically requires Rho-kinase (ROCK)-mediated myosin light chain (MLC) phosphorylation. Taken together, these data indicate that PINCH1 function at cell-cell adhesion sites is regulated by mechanical stress and this function seems independent of ILK and parvin.
How Changes in Cell Mechanical Properties Induce Cancerous Behavior

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Advances in biophysical techniques have made it possible to observe and identify the differences between the mechanical properties of healthy and cancerous cells. It has been suggested that these changes play an important role in uncontrolled growth and proliferation of cancer cells. How and to what extent these mechanical changes induce and influence cancerous behavior is unclear. Using three dimensional discrete computational models based purely on the mechanical interactions between cells, we show that increase in the compliance of a few mutated cells, as observed for cancer cells, can cause them to grow at a much faster rate compared to surrounding healthy cells. The simulations also show that an increase in inter-cellular adhesion amongst mutant cells results in rapidly growing compact malignant tumors while a decrease in the mutant cell adhesivity results in more spread-out, finger-like, stunted tumors. These observations can be qualitatively compared to a number of experimental and clinical observations. The model helps bridge the gap between the numerous interdependent biochemical carcinogenic factors and the physical manifestation of tumor growth and proliferation by providing a mechanistic pathway for cancerous behavior of cells.
Quantification of Traction Applied by Epithelial Cell Clusters

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Multicellular assembly of epithelial cells involves adhesion of individual cells to the underlying substrate via cell-substrate adhesions and their neighbors via direct cell-cell interaction. For these cells to assemble and adhere, mechanical forces must be present. The mechanical interaction between cells has been shown to affect cell growth, adhesion, and migration. Here, we study the effects of substrate stiffness and epidermal growth factor on the distribution of tractions within an epithelial cell cluster. The tractions are measured by using three-dimensional traction force microscopy, which employs high-resolution confocal microscopy and digital volume correlation. A finite element model that simulates the contraction of a cluster of cells is developed to better understand the magnitude and distribution of tractions under varying conditions. The results are discussed in the context of cell spreading and proliferation.
Dependence of Nuclear Transport of Paxillin on Traction Forces

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Paxillin is a focal adhesion protein, influencing activities, including matrix organization, cytoskeleton regulation, and gene expression. Signals from the focal adhesion FA are key determinants of differentiation and other cellular processes. Translocation of focal adhesion molecules, like paxillin, zyxin and Hic-5, to the nucleus has been implicated in transcriptional control. We find that translocation of paxillin is dependent upon traction forces and contractile state of the cells. Disruption of the acto-myosin network leads to a drop in the amount of paxillin translocating to the nucleus. Changing the cell’s contractile state using fibronectin micro-patterns also shows differences in paxillin’s nuclear transport. These results imply that paxillin carries mechanosensory information from the focal adhesions to the nucleus and influence cell behavior.
Transitions in Nuclear Plasticity and Higher-Order Chromatin Assembly during Cellular Differentiation

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Undifferentiated cells integrate physico-chemical cues from the local microenvironment to elicit lineage specific gene expression programs. Recent evidence from other labs including ours suggests that undifferentiated cell nucleus is soft, chromatin bound proteins are mobile, comprise of bivalent histone modifications and a highly active transcriptome. However the underlying mechanisms are still unclear. In this study, using high resolution live-cell fluorescence polarization imaging, we analyze the spatio-temporal aspects of nuclear organization and chromatin structure in mouse embryonic stem (ES) cells and contrast them to primary embryonic fibroblast (PMEF) cells. Higher-order chromatin compaction states exhibit unique features in ES cells, marked by homogeneous chromatin compaction but heterogeneity at the population level, but PMEFs evidence an inverse correlation. This heterogeneous profile of chromatin compaction is an active ATP dependent phenomenon. In addition, the nuclear lamina and actin cytoskeleton is highly flexible in ES cells but are frozen in PMEFs. Further the temporal evolution of chromatin plasticity is studied in in vivo developing Drosophila embryos. Taken together these results suggest that ES cells exhibit a broad epigenetic landscape transitioning into a frozen configuration in higher-order chromatin assembly and positioning – as lineage specific gene expression programs emerge.
Self-Organization of Cytoplasmic Actin-Myosin-Formin Network

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In eukaryotic cells, specialized actomyosin structures such as stress fibers and cytokinetic contractile rings are well known, but there is very limited understanding of the cytoplasmic actomyosin network. To elucidate this actomyosin network organization, we perturb it using Latrunculin A (LA). This treatment led to emergence of asters with diameter of 0.5-1.2µm, visualized by fluorescently tagged Lifeact or beta-actin in regions of cytoplasm not associated with remaining stress fiber or focal adhesions. These asters were very dynamic moving vigorously, often fusing with each other. Using dual color TIRF microscopy live imaging, myosin clusters were found either between or co-localized with actin aster centers, while filamin A and formin protein DAAM1 co-localized with the centers. Notably, PALM microscopy revealed that even in non-treated cells DAAM1 was localized in patches spaced similarly to the asters that emerged after the LA treatment. Actin aster movements induced by LA can be stopped by adding blebbistatin, a myosin II inhibitor, or largely reduced by treatment with formin inhibitor SMIFH2. On the other hand, in filamin A-/- cells, LA treatment resulted in formation of asters moving more rapidly and fusing and splitting more frequently than in control cells. Re-introduction of filamin A into the knockout cells decreased the asters’ dynamics significantly. Increasing the level of filamin A in the control cells caused the reduced velocity or complete block of the aster motility. We developed a computational model which, in agreement with these data, suggests that myosin motors slide actin filaments into the multiple asters with centers stabilized by cross-linkers and reinforced by formin-mediated nucleation, while myosin is stabilized at the edges between the asters where its contractile action causes aster movements. Modeling suggests that the dynamic-aster mode of self-organization is optimal for mechanical connectedness of the cytoplasm in perturbed actin networks.
Myosin Driven Actomyosin Ring Contraction In Vitro

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Cytokinesis in many eukaryotes involves the closure of an actomyosin based contractile ring. The relative contributions of actin polymerization, disassembly and myosin II function in ring closure are not fully understood. Here we establish an in vitro system for the study of actomyosin ring disassembly and closure in the fission yeast Schizaosaccharomyces pombe. We show that fission yeast cells lacking cell walls are capable of assembling actomyosin rings, which undergo rapid constriction upon cell permeabilization and ATP addition. The constriction of these rings was inhibited upon addition of the type II myosin inhibitor Blebbistatin. Surprisingly, neither actin polymerization nor disassembly was required for closure of actomyosin rings. Interestingly, addition of endogenous and exogenous actin cross-linking proteins blocked ring constriction. These studies establish that myosin II function and down regulation of actin cross-linking are sufficient for the mechanics of actomyosin ring closure.
Physical Model for Self Organization of Actin Cytoskeleton and Adhesion Complexes at the Cell Leading Edge

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Cell motion is driven by interplay between the actin cytoskeleton and the cell adhesion complexes in the front part of the cell. The actin network undergoes retrograde flow and, at the same time, exhibits a distinctive spatial organization. It segregates into lamellipodium, a narrow band of dense actin gel adjacent to the cell edge, and lamellum, which spreads towards the cell interior and is filled by a sparse actin network. The lamellipodium and lamellum are separated by a well-defined boundary of a characteristic shape. The adhesion complexes are non-uniformly distributed such that the nascent adhesions form underneath the lamellipodium whereas the mature complexes concentrate at the lamellipodium-lamellum boundary and underlie the lamellum. Here we suggest a physical model for this characteristic organization of the actin-adhesion system. The model is based on the ability of the adhesion complexes to sense mechanical forces, the stick-slip character of the interaction between the adhesions and the moving actin network, and a hypothetical propensity of the actin network to disintegrate upon sufficiently strong stretching stresses. We numerically analyze the system evolution and identify three possible types of its steady-state organization, all observed in living cells: two states in which the actin networks exhibit segregation into lamellipodium and lamellum whereas the cell edge either remains stationary or moves, and a state where the actin network does not undergo segregation. The crucial parameter determining the type of the steady state is the rate of generation of new adhesion complexes. Moreover, the model recovers and suggests physical mechanisms of more delicate dynamic features of the cell edge behavior: the asynchronous fluctuations and outward bulging of the edge, and the dependence of the edge protrusion velocity on the rate of the nascent adhesion generation. Finally, the model predicts formation of precursors of the actin stress fibers.
Stretch-Induced Remodeling of Actin Filaments and Adherence Junction Proteins in NIH3T3 Cell Sheets

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Focal adhesions and actin stress fibers are the key structures in mechano-sensing in cells. Recent studies, however, showed that these structures are not present on soft substrate, suggesting that other mechano-sensing mechanism(s) are present inside soft tissues. For instance, it is probable that cell-cell junction proteins play important roles when cells are not attached to substrate. Here we studied the effect of mechanical stretch on NIH 3T3 cells that are not adhered to the surface. As the sheet of cells was prepared without enzymatic digestion, artificial tissue like structure was obtained with intact cell-cell adhesion maintained. Before the stretch, RFP-actin was not observed under the real-time confocal microscope. When cells were stretched, fiber structure of actin filaments became visible parallel to the direction of the stretch. Localization of GFP-β-catenin was observed as patchy structures, which was not present when cells were not under tension, either. These results indicate that the remodeling of actin filament and adherence junction proteins are induced by mechanical stimuli.
Micro-Fabricated Substrates to Study Mechanotransduction

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We have developed an original approach based on micro-fabricated substrates of PolyDimethyl-Siloxane (PDMS) to study cell migration. We used a closely spaced array of flexible micropillars of different sizes to measure the forces exerted by cells on their substrates and to modify the effective rigidity of the substrate. In particular, we performed durotaxis experiments using substrates with a well defined frontier between two regions of different rigidities. We observed that a cell arriving to the frontier is more inclined to go to the more rigid region than to the softer one. We propose also to analyze the cell response to an external applied stress by a well-controlled actuation of the substrate. To do so, we developed magnetic pillars. Using polyacrylamide hydrogels doped with ferromagnetic iron oxide particles or ferrofluids, we can make magnetic pillars with diameters of 4 to 10 µm while a magnetic field can be locally applied with a magnetic needle. With such a technique we can exert forces of the range of several nN. Those substrates can be helpful to study the mechanical response of cells to an external force or to local changes in their microenvironment.
Turnover Dynamics of p130Cas at Focal Adhesions

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There is accumulating evidence that mechanically induced signal transduction, termed as mechatransduction, is involved in the regulation of a variety of physiological cell functions. Since focal adhesions (FAs) are the sites where actomyosin-derived tensile forces are concentrated, FA proteins are thought to play significant roles in mechatransensing.

We recently reported that the FA protein p130Cas (Cas: Crk-associated substrate) acts as a force-sensor through mechanical extension-dependent phosphorylation (Sawada et al., 2006, Cell). To further define the role of Cas in the mechatransensation, we analyzed the dynamic localization and turnover of Cas molecules at FAs. Time-lapse fluorescence imaging of mCherry-paxillin demonstrated longer lifetime of FAs in Cas-deficient mouse embryonic fibroblasts (MEFs), compared with those in the control MEFs. FA lifetime was completely rescued by the expression of exogenous Cas (wild type, WT), but not by the expression of the phosphorylation-defective and the Src-binding (SB)-attenuated mutants of Cas, Cas15YF and CasmPR, respectively. Considering the significant decrease in the phosphorylation of CasmPR compared with CasWT, these results suggest that phosphorylation of Cas is involved in the FA dynamics.

Both the N-terminal SH3- and C-terminal SB-domains have been reported to be required for Cas to be localized to FAs (Nakamoto et al, 1997, Mol Cell Biol), suggesting that Cas molecules are anchored to FA complex via those two domains. Although the lifetime of individual FAs was longer than 10 minutes in MEFs, the fluorescence recovery after photobleaching (FRAP) analysis showed that the average recovery time of GFP-CasWT at FAs was shorter than 30 seconds. These results suggest that Cas molecules are constantly subject to rapid turnover within the individual FAs. Furthermore, FRAP analysis using Cas-deficient cells demonstrated lower recovery rate and longer recovery time of GFP-Cas15YF compared with GFP-CasWT, suggesting that release of Cas molecules from FAs depends upon their phosphorylation status. In addition, we found that the recovery time of GFP-CasmPR was not significantly shorter, despite the tempered SB-domain anchoring to FA complex, than that of GFP-CasWT. These results suggest that the disruption of the SH3-domain anchor of Cas to FA complex is dependent on the phosphorylation status of Cas. Together with the exclusive localization of phosphorylated Cas at FAs, phosphatase(s) of Cas may regulate the disruption of the association of Cas SH3-domain with the FA complex.

Thus, the mechatransensing protein Cas plays a significant role in the regulation of FA dynamics through its phosphorylation-dependent rapid turnover.
Tension is Insufficient to Drive Focal Adhesion Maturation in the Absence of a Dorsal Stress Fiber Template

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Myosin-II mediated focal adhesion maturation is crucial to modulate physical and biochemical signaling between cells and the ECM. Since myosin drives both dorsal stress fiber assembly and enhanced tension at adhesions simultaneously, the extent to which focal adhesion maturation is driven by tension or changes in actin architecture is unknown. We find that force transmission to adhesions can occur in the absence of prominent dorsal stress fibers linking adhesions to lamellar actin. Force-mediated cues are sufficient to stabilize nascent adhesions to the ECM and facilitate adhesion dynamics. However, force-mediated adhesion signaling is not sufficient to mediate classic hallmarks of adhesion maturation including the accumulation of phosphorylated paxillin and FAK and the formation of fibrillar adhesions required for fibronectin remodeling. For the compositional and morphological maturation of adhesions, dorsal stress fiber assembly at the adhesion plaque must occur. Thus, myosin-mediated mechanotransduction pathways are insufficient to drive essential aspects of focal adhesion maturation in the absence of a dorsal stress fiber template.
Soft ECM Triggering Integrin Activation and Internalization Play a Role in ECM Stiffness-Induced Mesenchymal Stem Cell Lineage Specification

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Background: There are many evidences demonstrating that the mechanical properties (e.g., elasticity) of adhesion substrates modulate stem cell fate in both two-dimensional (2D) (1-2) and three-dimensional (3D) (3) cultures. However, the mechanism by which the mechanical properties of extracellular matrix (ECM) trigger the chemical signaling processes has not been clearly understood. Integrins are well-documented mechanosensors positioning at the beginning of the sensing pathway. β1 integrin is an important integrin subunit in bone marrow mesenchymal stem cells (BMMSCs) (4). In the present study, we aimed to explore the ECM elasticity-sensing mechanism of BMMSCs, especially the role of β1 integrin.

Methods and techniques: Primary BMMSCs were harvested from one-month-old Sprague Dawley rats and cultured in DMEM-F12 medium with the addition of 10% FBS, 2 mmol/L L-glutamine and 1% penicillin-streptomycin at 37°C and 5% CO₂. Immunocytochemical staining and western blotting was carried out with an antibody recognizing the active conformation of β1 integrin to assay the β1 integrin activation. Biotin labeling of cell surface proteins was used to evaluate the integrin internalization in BMMSCs on substrate of different elasticity.

Main results: We have observed that β1 integrin activation in bone marrow mesenchymal stem cells (BMMSCs) was induced by soft substrate to a significantly greater degree than stiff substrate. In contrast, however, the level of cell surface integrin on soft substrate was significantly lower than that on stiff substrate. Soft substrate markedly enhanced the internalization of integrin, and this was mediated mainly through caveolae/raft-dependent endocytosis. The inhibition of integrin internalization blocked the neural linage specification of BMMSCs on soft substrate. Furthermore, soft substrate also repressed the bone morphogenetic protein (BMP)/Smad pathway at least partially through integrin-regulated BMP receptor endocytosis. A measurement of the rupture force of β1 integrin-RDG bond by atomic force microscope indicate that integrin-ligand complexes are more easily ruptured on soft substrate; this may contribute to the enhancement of integrin internalization on soft substrate.

Conclusions: Taken together, our results suggest that ECM elasticity affects integrin activity and trafficking to modulate integrin BMP receptor internalization, thus contributing to stem cell lineage specification.

Regulation of Neuronal Initiation and Maturation by BPGAP1 and Ras Signaling

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Proper development of a neuron involves several processes of initiation, outgrowth, differentiation, axon pathfinding and maintenance. Small GTPases and its regulators such as GAPs and GEFs play important roles in neuronal development. We have shown that the RhoGAP, BPGAP1 (BNIP-2 and Cdc42GAP Homology domain containing, Proline-rich and Cdc42GAP-like protein subtype-1), regulates cell migration and ERK signaling by the concerted action of its multiple domains interacting with cortactin, endophilin, Mek2, Pin1. Using the PC12 neuronal differentiation model, we have shown that BPGAP1 constitutively activates the Ras signaling pathway downstream of epidermal growth factor stimulation to initiate and promote neurite outgrowth and differentiation, in a process further regulated by the interplay of selected activators and suppressors of Ras signaling. Our current study also reveals one novel interacting partner of BPGAP1 that serves as a negative Ras regulator. This protein inhibits the constitutive Ras activation and subsequent differentiation of PC12 cells. Using live imaging to track the differentiation of PC12 cells, we have established that the interaction of BPGAP1 and its novel interacting protein affects not only the initiation of neurite outgrowth but also growth cone maturation and dynamics. The significance of BPGAP1 and this novel regulator in influencing Ras signaling during neuronal development will be discussed.
High Speed Scanless Fluorescent Nanoscopy by Modulated Pupil Function Subtraction

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Superresolution fluorescent microscopy (nanoscopy) offers unprecedented resolution of biological structures by overcoming the fundamental diffraction limit of light. Here, we have developed novel nanoscopy that achieves 120 nm and 500 Hz resolutions by obtaining a subtracted image from two images whose pupil functions are independently modulated. We call this technique DiMPS (Distinct optical Modulated Pupil function Subtractive imaging). Such high temporal resolution was made possible because DiMPS acquires a superresolution image within one frame of a camera. Because DiMPS is compatible with fluorescent probes that lack polarization or coherency, we can apply it to various biological investigations. In this paper, we describe basic DiMPS principles and constructions, and demonstrate the potentials of DiMPS for live cell imaging both on the membrane and intracellular, and for single particle tracking by using E-cadherin as our model. We found E-cadherin localized periodically every ~200 nm, indicating it is most likely responsible for actin mesh scaffolding.
Hepatocyte Growth Factor Acutely Perturbs Actomyosin Driven E-cadherin Dynamics

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E-cadherin mediated cell-cell adhesion is critical for maintaining epithelial integrity, furthermore its dysregulation is recognized as a prerequisite for epithelial cancers to invade and metastasize. Recent advances have emphasized E-cadherin’s role in cell-cell adhesion via cooperation with the myosins, which are actin-based motor proteins. Despite this, myosin driven cadherin dynamics and its dysregulation in disease context is poorly understood. To address this, new model systems are established, endogenous E-cadherin in Caco-2 colorectal cell lines are replaced with fluorescence tagged E-cadherin. In this model, E-cadherin homophilic adhesion foci at the lateral cell-cell interface exhibits pulsed contractions even at steady state.

Employing both pharmacological inhibition of Myosin II and acute treatment with Hepatocyte Growth Factor (HGF) not only fragments the Epithelial Zonula Adherens but also reduces pulsation of E-cadherin clusters at basolateral regions of epithelial junctions. Taken together, my data supports the model that Myosin II driven E-cadherin contractile flow is required for cell-cell interactions and homeostasis of the epithelial zonula adherens.
Viscoelastic Substrate Elicits Collective Migration Through Cytoskeletal Reorganization

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Collective cell migration is governed by coordination of cell-cell and cell-matrix adhesions, but the mechanism that regulates reciprocal regulation of adhesions is unknown. We have recently found a critical rheological property of cell substrates, upon transition from highly elastic to visco-elastic substrates, induces highly correlated and E-cadherin-dependent cell movement of epithelial monolayers. How cells convey mechano-signal from the matrix to adherens junctions and accordingly migrate in a concerted fashion is the main focus of current study. Twelve inhibitors have been used to screen cellular components that are essential for viscoelastic substrate-elicited phenotypes. Our results strongly argue that actin dynamics and actomyosin activity are indispensable for coordinated migration, while PI3K, Src family kinases, β-catenin stability, and microtubule dynamics are possibly involved. Actin organization has subsequently been studied by 3D confocal microscopy in epithelial sheets attached to viscoelastic substrates. Deconvolved confocal images reveal that stress fibers (SFs) make little connection to substrate-coating fibronectin but lie along the ventral surface of cells on viscoelastic substrates; on the other hand, long SFs are formed and anchored to fibronectin at peripheral adhesions on stiff substrates. In addition, vinculin-containing adhesions associated with SFs are found predominantly at center of the basal surface on viscoelastic substrates, but have accumulated at and thus outlined the cell boundary on stiff matrix. Taken together, viscoelastic matrix has induced reorganization of SFs and cell-matrix adhesions that may constitute the mechanistic basis for adherens junction-dependent collective migration.
Collective Behavior of Epithelial and Metastatic Cells - Through the Third Dimension

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Cell migration contributes to numerous biological functions, from the establishment of the body plan to immunological responses. It also impacts health since disorganized cell movements, acquisition of migrating capacity by cells that should remain still, or loss of motility in normally motile cells, have dramatic consequences in a variety of situations from embryo lethality to diseases including cancer. It has been demonstrated that many features of cells migrating from tumors are mimicked.

Recent evidence has outlined the fundamental differences between the conventional 2D cell migration on flat rigid substrates and the 3D migration in collagen gels. However the importance of an out-of-plane curvature on a 2D epithelium has hardly been studied so far although many epithelial bidimensional tissues exhibit such an intrinsic curvature which can become very important in 3D morphogenetic processes.

We tried to take this third dimension into account by studying the importance of an out-of-plane curvature on migration and growth of epithelial cells. Our current work is to study the collective behavior of prostate cancer cells inside well-controlled 3D microenvironments. The aim of our present project is to determine the phenotypic changes in metastatic cancer cells that allow it to attack normal tissues.
Mechanical Properties of Bleb Formation during Cell Migration of Primordial Germ Cells in Ze41 Zebrafish Embryos

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Whereas the mechanisms controlling single-cell migration in vitro are relatively well understood, less is known concerning the mechanisms promoting the motility of individual cells in vivo. In particular, it is not clear how cells that form blebs in their migration polarize to bring about movement in the context of the three-dimensional cellular environment. Understanding the means by which cells move within the tissue would benefit from determining the forces they exert on the environment and from evaluating the intracellular forces employed when moving the cell cytoplasm forward.

To this end, we have implemented holographic optical tweezers (HOT) in a fluorescence microscope to probe the mechanical properties of primordial germ cells (PGCs) of zebrafishes in-vivo. Calibrating and optimizing the system, we managed to probe PGCs that migrate in relatively deep locations, a few cell layers beneath the surface of the embryo. The HOT setup will allow us to investigate the rigidity of the cell at multiple positions simultaneously, to analyse the spatial and temporal evolution and changes in this parameter during blebbing, and to evaluate the force generated by cytoplasmic streaming in the course of protrusion formation.
Mesenchymal-epithelial transition (MET) is a fundamental process for cancer metastasis, induced pluripotent stem cell reprogramming and segmentation of the somite during embryonic development. It has been shown by Nakaya (2004) that the Rho GTPase Cdc42 plays an important role in MET to affect somital segmentation in chick. In our lab, we are interested in the Cdc42 binding kinases MRCKs and shown that together with their partners Leucine rich adaptor protein LRAP35a form a triple complex with myosin II-related MYO18A in the actomyosin retrograde flow during cell migration (Tan, 2008). The on-going projects are to understand the biological meaning of these interactions by using animal models including mouse and zebrafish. In zebrafish, we noticed that mrckb and lrap35b were expressed synergistically during somital segmentation, and our preliminary data suggest that they play an important role in MET during somital segmentation in a similar manner with that of cdc42. Our future works will focus on the molecular regulation of these proteins in MET.
Caveolae are invaginations of the plasma membrane, which can constitute up to 60% of the total cellular surface area. We have recently demonstrated that caveolae work as physiological membrane reservoirs that quickly disassemble under cellular swelling (Sinha et al., 2011). We have now extended our findings and show that caveolae-null cells are more sensitive to hypo-osmotically increased membrane tension. Making use of FLIM/FRET, we show in real time that caveolin and cavin-1, whose interaction regulates caveola biogenesis, dynamically and reversibly, respond to membrane tension variations through dissociation and re-association. Once dissociated from cavin-1, caveolin is internalized in late endosomes and lysosomes through a dynamin-independent pathway. Cavin-1, on the other hand, is found diffused in the cytosol and from there it translocates to the nuclear compartment. Studies with truncated forms of cavin-1 indicate that C-terminal domains target the protein to the nucleus whilst N-terminal domains retain the protein in the cytoplasm. Altogether, our results indicate that, besides the elementary benefit of providing extra surface area, the flattening of caveolae and the dissociation of the caveolin and cavin-1 proteins could release these proteins for modulating a cellular response at the molecular level in response to mechanical stimuli.

Reference
Podosome Rings Generate Forces that Drive Saltatory Osteoclast Migration

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Podosomes are dynamic, actin-containing adhesion structures that collectively self-organize as rings. In this study, we first show by observing osteoclasts plated on bead-seeded soft substrates that podosome assemblies, such as rings, are involved in tension forces. During the expansion of a podosome ring, substrate displacement is oriented outward, suggesting that podosomal structures push the substrate away. To further elucidate the function of forces generated by podosomes, we analyze osteoclast migration. Determining the centers of mass of the whole cell (G) and of actin (P), we demonstrate that osteoclasts migrate by “jumps” and that the trajectories of G and P are strongly correlated. The velocity of the center of mass as a function of time reveals that osteoclasts rapidly catch up with podosomal structures in a periodic pattern. We conclude that actin dynamics inside the cell are not only correlated with cell migration, but drive it.
Yield Strain of Human Erythrocytes Membrane for Impulsive Stretching

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The deformability of cells without its rupture is an important mechanical property. In particular red blood cells need to deform considerably while flowing through small capillaries. It has been confirmed that the RBC membrane can withstand a finite strain, beyond which it ruptures. The classical and very small yield areal strain of 2-4% for RBCs has been measured using micropipette aspiration by Evans et al in 1976. However, Leverett et al noted already in 1972 that this threshold is a function of the exposure duration, and may increase dramatically for shorter durations of the forcing.

Here, we quantify the yield strain for RBC membrane rupture using an impulse like forcing. Therefore, a collection of RBCs are stretched by the fast and transient flow created by a single laser-induced cavitation bubble. The duration of the fluid flow is in the tens of microseconds. The deformation of the cells is captured by a high speed camera and viability is monitored with fluorescence microscopy successively. We find that the probability of cell survival is closely related to the initial strain. Yet, the threshold linear and areal strain for membrane failure is much larger as compared to micropipette aspiration. Membrane integrity is assessed by the diffusion of fluorophores from the cell’s cytoplasm.

Key words: Human erythrocytes, Membrane, Rheology.
Mechanical Regulation of Auxin-Mediated Growth in Tomato Shoot Apex

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Plant growth is regulated by the phytohormone auxin and mechanics. Auxin accumulation and cell wall loosening are entangled in most growth regulation mechanisms; however, exactly how mechanics influences auxin is unknown. We show that developmentally relevant degree of mechanical strains can regulate auxin transport and accumulation. Osmotic treatment, external force application, and modulation of plasma membrane properties collectively show that the amount and intracellular localisation of the auxin efflux carrier PIN1 are sensitive to mechanical alterations. Modulation of the membrane alone was sufficient for the effects, and thus a model is formulated that the plasma membrane senses tissue mechanical strain via membrane tension, influencing vesicle trafficking directly and triggering mechano-transduction and responses. One implication of this fundamental mechanism is the mechanical amplification of auxin-mediated growth in young organ primordia. Growth-induced mechanical strains enhance PIN1 function and auxin accumulation, thereby prompting further growth, in a robust positive feedback loop.
N- Terminal and C-Terminal Domains of p140Cap can Inhibit Tumour Growth in a Variety of Cancer Cell Lines

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Recent research have identified the adaptor protein p140Cap suppressing the tumour cell properties by regulating Csk and Src kinase activity. In order to search for the domain of p140Cap which can mimic the properties of full length p140Cap in tumour suppression, we generated stable MDA-MB-231 breast cancer cells that express tyrosine rich fragment (NT) and C terminal domain (CT) as well as full length p140Cap. We found that the C-terminal domain which includes proline rich regions as well as the tyrosine rich domain which contains the Csk binding region down-regulate in vitro migration, proliferation, and suppress in vivo tumour growth at the same extent of the full length p140Cap. Moreover in these cells lines, we found a decreased phosphorylation of signalling molecules involved in cell survival and cell proliferation such as Src, FAK and Akt and decreased amount of Vimentin. Similar results were obtained in lung A549 and colon HT29 cancer cell lines. Interestingly in these two other cell types full length, C-terminal and N-terminal domains were able to down-regulate in vivo tumour progression and phosphorylation of Src, FAK and Akt in A549 while in HT29 full length as well as both the domain can downregulate phosphorylation of FAK, Akt and ERK. Overall these results indicate that p140Cap as well as its domains inhibit growth of a variety of cancer cells, highlighting their possible to use as therapeutic molecules.
Crosstalk Between Ras and RhoA Signaling Regulate Liver Development and Hepatocellular Carcinomas in a Zebrafish Cancer Model

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Ras and Rho small GTPases are key molecular switches that control cell dynamics, cell growth and tissue development through their distinct signaling pathways. While much has been learnt about their individual functions in both cells and whole-animals, the physiological/pathophysiological consequences of their signaling crosstalk in multi-cellular context in-vivo remains unknown, especially in liver development and liver cancers. Furthermore, the roles of rhoA in ras-mediated transformation and their crosstalk in-vitro remain controversial. Capitalizing on the growing importance and feasibility of zebrafish Danio rerio as an alternate cancer model, we have generated liver-specific tet-on inducible transgenic lines expressing oncogenic Kras G12V, rhoA, constitutively-active rhoA G14V or dominant-negative rhoA T19N. Double transgenic lines of oncogenic Kras and rhoA or its mutants were generated by crossing the selected lines. Based on quantitative bioimaging and molecular markers for genetic and signaling aberrations, we show that induced expression of oncogenic Kras during early development could lead to liver enlargement, concomitant with elevated Raf/MAPK and PI3K/AKT signaling. This effect was augmented by dominant-negative rhoA, but was significantly reduced by the constitutively-active rhoA. Induced expression of oncogenic Kras in adult transgenic fishes led to the development of hepatocellular adenomas (HA) and/or hepatocellular carcinomas (HCC). However, despite homogenous expression of active Kras in the entire liver, the ERK signaling was more active in cells present in the periphery of the liver compared to its center, indicative of possible mechanical/physical influences on growth-related ERK signaling. Survival studies further revealed that co-expression of dominant-negative rhoA with oncogenic Kras increased the mortality rate significantly, as compared to the other single or double transgenic lines. These results therefore represent the first in-vivo and tissue-specific vertebrate model of hepatocellular transformation and reveal herein a previously unappreciated level of signaling crosstalk between ras and rhoA in regulating hepatocytes transformation in-vivo. The significance of this will be discussed.
Heart Extracellular Matrix Supports Cardiomyocyte Differentiation of Mouse Embryonic Stem Cells

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We have evaluated the effect of heart extracellular matrix (ECM) on the cardiomyocyte differentiation of mouse embryonic stem cells (ES cells) using de-cellularized heart tissue. We found that there are substantial differences in the protein content of SDS treated heart tissue compared to that of liver tissue assessed by mass-spectroscopy. When mouse ES cells were cultured on thin (60 μm) sections of de-cellularized heart tissue, expressions of cardiac genes was examined by quantitative real-time PCR analysis which showed that cMHC (p < 0.1) and cardiac troponin I (cTnI) (p < 0.05) were highly expressed in ES cells cultured on heart ECM compared with those on liver ECM. Addition, the protein expression of cardiac myosin heavy chain (cMHC) and cTnI were detected in cells on the heart ECM after 2 weeks. On the other hand, ES cells on liver ECM did not express cMHC and cTnI. Taken together, these results suggested that heart ECM plays a critical role in cardiomyocyte differentiation of ES cells. It is suggested that tissue specific ECM involved in cell linage specification through mechanotransduction derived from a space, elasticity and components.
Effective Neurogenesis of Human Adipose Tissue-Derived Stem Cells by Transient Aggregate Culture using Microfluidic System

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Human adipose tissue-derived stem cells (hADSCs) have great potentials in regenerative medicines due to its active growth and differentiation. ADSC is able to reversibly de-differentiate and transdifferentiate in response to environmental factors. Here, we report a microfluidic system to construct the artificial microenvironment to enhance the plasticity of the hADSCs. Spheres from hADSCs were trapped by micropillars in the microfluidic channel and cultured in neural induction medium. Cells were grown in the aggregate forms for 3 days, while they experienced cell-cell interactions and hypoxia, thereby inducing HIF1α expression. During this period, the expressions of stemness markers Oct4 and Nanog also prominently increased. After 3 days, cells migrated out of the aggregates and started neural differentiation. After 7 days, cells presented neuron-like morphologies and expressed early neuronal marker TuJ-1. After 14 days, 60% of the cells were differentiated into GABA+ neurons, while only 30% of cells in culture dishes were differentiated into GABA+ neurons. These results suggest that aggregate culture in our system generate hypoxic microenvironment where HIF 1α and Oct4 expressions can be enhanced, leading to the effective transdifferentiation of the stem cells into neurons. These results demonstrated that the microfluidic system is highly useful for engineering stem cell niches.

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Mechanosensation in both Body and Nose is required for C. elegans to Sense and Navigate through Microfabricated Funnels

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Mechanosensation is the process by which mechanical stimuli including touch and pain are converted into neuronal signals. The process of mechanosensation has been extensively studied in the nematode C. elegans. The five neurons (ALML, ALMR, AVM, PLML, and PLMR) sense gentle body touch, while as many as 20 neurons including ASH and FLP with sensory endings in or around the nose sense nose touch.

When a population of C. elegans was exposed to microfabricated funnels [1, 2], their random migration through the openings was rectified by tracking (trapping) of the crawling worms along the funnel wall. This led to a buildup of the density of worms on the narrow opening side of the funnel wall. Unlike the wild type (N2), the body touch mutants (mec-4 and mec-10) were often bounced back upon bumping to the wall and their motion were not rectified, while the nose touch mutant (osm-9) rapidly migrated along to the wall and was not properly trapped on the narrow opening side of the funnel wall. These results suggest that the navigation capability of worm depends on mechanosensation on both body and nose when they are exposed to obstacles.


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Feedback Control of Tissue Homeostasis by Activation of Cytokine- and Mechano-signalling in Adult Drosophila Intestinal Stem Cells In Vivo

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Biophysical experiments suggest that tension forces can trigger cell proliferation of cultured cell lines in vitro. The nature of mechanical cues in an organism in vivo as well as the molecular mechanism how a mechanical signal may be converted to a biochemical proliferative cue remain, however, largely unclear.

We have recently revealed that upd cytokine expression and release from dying differentiated enterocyte (EC) cells of the adult Drosophila midgut induces pro-proliferative JAK-STAT signaling in resident adult intestinal tissue stem cells (ISCs) in vivo, a feedback mechanism that explains how the number of cells in a tissue is maintained by coupling EC cell loss to regenerative ISC division.

Genetically screening for primary factors potentially sensing mechanical forces in the tissue upon EC cell death and loss from the midgut epithelium, and converting them to a growth stimulus, we reveal that genetic deactivation of the core Hippo pathway results in enhanced ISC proliferation rate in vivo. The Hippo signaling pathway is an evolutionary conserved organ size control module that restrains tissue growth by control of cell proliferation and cell survival during animal development. Conceptually, the pathway integrates information from cell-cell contact and cell cortex and cytoskeleton associated tumor suppressor factors that converge in inhibitory phosphorylation and cytoplasmic retention of the central downstream transcriptional co-activator Yorkie.

We show that genetic mutation of the core Hippo pathway kinase warts as well as direct activation of yki in the differentiated cells of the Drosophila midgut enhance cytokine expression, thereby triggering proliferation of neighbouring wildtype ISC stem cells. We further reveal that Yki transcriptional activity is triggered upon damage to the midgut tissue and that Yki function is essential for midgut tissue regeneration. We are testing how Yki activity is molecularly controlled by physical forces in the intestinal epithelium in vivo.
Microfluidic Device with Elastic Posts for Measurement of Contractile Force of Single Myotube

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The contractile force of multinucleated muscle fibers is a great indicator for evaluating muscular dystrophy. However, there is lack of an efficient and affordable device for quantifying the contractile force of muscle fibers in vitro. In this study, we describe a microfluidic cell culture device equipped with elastic posts for measuring contractile force generated by a single myotube in situ. The microfluidic device is composed of an array of microchannels with a dimension of 50 µm (width) x 500 µm (length) x 100 µm (height) having PDMS (polydimethyl siloxane) pillars with a dimension of 50 µm (diameter) x 100 µm (height) located at the both ends of each channel. When undifferentiated myoblast (C2C12) cells were cultured in the microchannels for several days, they were fused to form multinucleated single myotubes and then started to be attached to pillars in the channel. Since the contractile force of each myotube can be calculated from the bending degree of micropillars pulled by a single myotube, it is expected that our device can be useful tool for studying the mechanical characteristics of myotubes in vitro.

This research was supported by the BK21 (Brain Korea 21) program.
Forces, Strain Energy and Geometry of Keratinocyte Colonies

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It is well known that the contractility of actomyosin generates mechanical forces. To date, however, there have been limited studies on the mechanics of multicellular systems. It remains an open question how the addition of intercellular adhesions affects the generation of mechanical forces, both at the cell–cell interface and between cells and their extracellular matrix. This topic is particularly relevant in the mammalian epidermis, as maintenance of intercellular adhesions is essential to the mechanical integrity of the epithelium.

During the formation of calcium-dependent intercellular adhesions, epithelial cells reorganize their mechanical attributes, such as the force they exert on each other and on their underlying substrate. Also during this process, they rearrange their cellular organization, including cadherins, focal adhesions, and cytoskeleton. Using traction force microscopy analyses of primary mouse keratinocytes, we correlate the physics and biochemistry of adhesions and cytoskeleton during the differentiation of colonies of keratinocytes. We examine how mechanical properties scale with the size and geometry of the colony. Additionally, we apply various perturbations to the cadherins, desmosomes, and the extracellular matrix in order to determine the role that intercellular adhesion plays in the mechanical behavior and output of small tissues.
Computational Electro-Mechanics to Investigate Pathogenic Factors for Gastrointestinal Dysmotility

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Background
Recent evidences suggest non-neuronal sodium channel related mutations in causing unexplained gastrointestinal (GI) motility disorders. So far, HEK cells study characterized mutation induced alterations in sodium channel behaviour, but further work is required for a clearer link on these mutations as pathogenic factors.

Aims
Computational models were developed and/or used to investigate these mutations as pathogenic factors.

Methods
Motility involves electrics and mechanics of the GI smooth muscle cells (SMC) and interstitial Cajal cells (ICC). Markov models describing wild-type and mutation affected sodium channel electrics were developed and integrated into GI SMC and ICC electrical models. The predicted electrical results were input into a mechanical model of the GI SMC to evaluate mechanical consequences.

Results
The simulation results predicted alteration of both ICC and SMC electrics due to the mutations. Alterations in electrical behaviour changed calcium regulation. In turn, mechanical behaviour in terms of normalized SMC contractile force was affected.

Conclusion
The preliminary studies indicated the mutations’ ability to alter electromechanics in the GI context. However, more work is required to determine how pathogenic these changes are. One future direction is to build upon existing models for a well defined electromechanical or mechanoelectrical model.
Understanding Apical Constriction during Drosophila Dorsal Closure

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Cell shape changes and rearrangements drive different morphogenetic movements during development. These shape changes and rearrangements are known to influence and be influenced by mechanochemical cues.

I am currently investigating the cell shape changes that drive Dorsal closure in *Drosophila melanogaster*. This movement achieves the sealing of the dorsal side of the *Drosophila* embryo via the contraction of the tissue amnioserosa and elongation of the flanking epidermis. Amnioserosa (AS) contracts via constriction of individual cells. The AS cells employ novel and highly dynamic modes of constriction which we have uncovered using high resolution 4D confocal imaging. The AS cells display a biphasic mode of constriction wherein the first phase displays low net apical constriction and high apical area fluctuations. The second phase in contrast, shows net constriction and low fluctuations. I am currently exploring the dynamic cytoskeletal changes accompanying the AS cell constrictions via imaging of fluorescently tagged cytoskeletal elements and molecular motors and their differential role in achieving constriction. We have also identified mechanical and genetic cues which influence the apical constriction and its biphasic mode during dorsal closure.
Estimating the Dynamics of Forces During Morphogenesis

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It remains unclear how the rearrangement and packing of cells are promoted or constrained under the global balance of stress within a cell population/a tissue due to lack of proper force measurement methods. Here we develop a novel method for estimating cell pressure and the tension of cell adhesion surfaces in vivo. By using the method, we characterize the intrinsic cellular forces and the anisotropic external stretching force acting on the Drosophila pupal wing. We then address how the balance between intrinsic and extrinsic force regulates the formation of hexagonal cell array. Our quantification of local cell stress and of corresponding changes in cell geometry reveals that the stress direction of a cell population provides the directional information for the orientation of hexagonal cells to promote hexagonal cell packing. The described force estimation method will become a powerful tool for analyzing how the morphogenetic behaviors of individual cells are orchestrated by spatio-temporal dynamics of forces at cell population level.
Crucial Role for the Phosphorylation of the Mechano-Sensing Protein p130Cas in Myogenic Differentiation

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Muscle is one of the tissues most sensitive to mechanical stimulation (e.g. physical exercise), and myogenic differentiation is a multi-step event that is essential for the development, maintenance and repair of muscle tissues. While we previously showed that the Src family kinase p130Cas acts as an ion channel-independent mechano-sensor that converts physical information into a biochemical signal, p130Cas-deficient mice are reportedly embryonic lethal, exhibiting poorly developed heart, disorganization of myofibrils and disruption of Z-disks, suggesting that p130Cas is involved in myogenesis.

Here, using murine C2C12 myoblasts as a model system, we demonstrate a significant role of p130Cas in myogenic differentiation. When we examined the C2C12 myoblasts in which p130Cas expression was knocked down by a retrovirus-based shRNA vector (CasKD myoblasts), the myotube formation was significantly attenuated under the differentiating condition. This was further supported by the decreased expression of myogenic markers, such as myogenin, α-actin and myosin heavy chain, as well as the decreased transcriptional activity of the serum responsive factor (SRF). Furthermore, we found that actin stress fiber formation was reduced with enhanced activity of cofilin, an F-actin severing protein, in CasKD myoblasts under the differentiating condition. Consistently, we observed, in those cells, an increase in G-/F-actin ratio and a decrease in the nuclear localization of MAL, a transcriptional cofactor of SRF, which has been reported to be impeded by the binding to G-actin.

The restoration of myogenic differentiation of CasKD myoblasts by re-introduction of wild-type p130Cas, but not its 15F mutant, indicates the importance of phosphorylation of p130Cas. In addition, myogenic differentiation and MAL nuclear localization were partially rescued in CasKD myoblasts when phospho-mimetic cofilin mutant (S3E) was introduced exogenously. Taken together, we conclude that p130Cas positively regulates myogenic differentiation by inactivating cofilin that negatively influences transcription of myogenic markers.
**Thrombospondin-1 induces Sinusoidal Endothelial Cell Defenestration**

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**Background:** Liver fibrosis is characterized by excessive accumulation of extracellular matrix (ECM), defenestration of sinusoidal endothelial cells (SECs), and high levels of fibrotic cytokines. The glycoprotein thrombospondin-1 (TSP1) has emerged as a target for anti-fibrotic therapy because of its importance in activating the fibrotic cytokine TGF-β1$^{[1]}$. Meanwhile, plasmin is a liver-secreted protease that has anti-fibrotic effects including ECM remodelling and cleavage of TSP1$^{[2]}$. In this project, we tested whether TSP1 has any direct effects on capillarization of the sinusoids, and whether any TSP1-induced effects would be reversed by plasmin.

**Materials/Methods:** Rat primary SECs were cultured and SEC defenestration was quantified using specific membrane markers and scanning electron microscopy.

**Results:** Treatment of primary rat SECs with recombinant TSP1 caused dose-dependent defenestration, with increased levels of basal membrane markers CD31, CD44 and vWF, and significant decreases in both porosity and average diameter of fenestration. TSP1-induced defenestration was reversed when cells were further treated with recombinant plasmin (PLS), which caused significant cleavage of TSP1. On-going experiments are studying the roles of TSP1 receptors (CD36 and CD47) and downstream cytoskeletal effectors (RhoA and MLCK) in the mechanism of defenestration.

**Conclusion:** SEC capillarization has been shown to precede portal hypertension, atherosclerosis and liver fibrosis development. The induction of defenestration by TSP1 in vitro augments the importance of TSP1 as a far-reaching, multi-functional target for anti-fibrotic therapies. Our work also suggests the importance of natural regulators of TSP1 such as plasmin for inducing regression of sinusoidal capillarization and fibrosis.

Tissue Driven Control of Oriented Cell Divisions in Neural Tube Morphogenesis

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How control of subcellular events in single cells determines morphogenesis on the scale of the tissue is largely unresolved. The stereotyped cross-midline mitoses of progenitors in the zebrafish neural keel provide a unique experimental paradigm for defining the role and control of single-cell orientation for tissue-level morphogenesis in vivo. We show here that the coordinated orientation of individual progenitor cell division in the neural keel is the cellular determinant required for morphogenesis into a neural tube epithelium with a single straight lumen. We find that Scribble is required for oriented cell division and that its function in this process is independent of canonical apicobasal and planar polarity pathways. We identify a role for Scribble in controlling clustering of α-catenin foci in dividing progenitors. Loss of either Scrib or N-cadherin results in abnormally orientated mitoses, reduced cross-midline cell divisions, and similar neural tube defects. We propose that Scribble-dependent nascent cell-cell adhesion clusters between neuroepithelial progenitors contribute to define orientation of their cell division. Finally, our data demonstrate that while oriented mitoses of individual cells determine neural tube architecture, the tissue can in turn feed back on its constituent cells to define their polarization and cell division orientation to ensure robust tissue morphogenesis.
Imaging Protein Activity in Live Embryos Using Fluorescence Resonance Energy Transfer (FRET) Biosensors

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Fluorescence resonance energy transfer (FRET)-based molecular biosensors serve as important tools for studying protein activity in live cells and have been widely used for this purpose in the past decade. However, FRET biosensors are rarely utilized in the context of the live organism. This is due to the inherent high cellular complexity and imaging challenges associated with the three-dimensional environment. Therefore, experimenters have to deal with low signal-to-noise ratios, extensive bleaching and with changing fluorescence properties dependent on the developmental stage of the organism. Here, we present an easy to use guide that will help even inexperienced experimenters to setup FRET measurements based on single-chain intra-molecular biosensors in the context of early embryonic development. We provide a general protocol for FRET ratio imaging in live embryos, including the data-acquisition conditions and the algorithm for ratio image generation. As example, we employ constructs reporting on the Rho GTPases activity level in zebrafish embryo. We then show the data acquired with pRaichu RacFRET biosensor to exemplify the adaptation and optimization of a particular biosensor for use in live embryos. This includes the choice of an optimal donor and acceptor pair, as well as the relevant controls that are necessary to establish the range between the active and inactive states of these biosensors.
Differential Mechanical Stability of Filamin A Rod Segments and Domain Pair Interaction

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Filamin A (FLNa), an actin cross-linking protein, consists of two subunits that dimerize through C-terminal self-association domain. Each subunit contains an N-terminal spectrin-related actin-binding domain followed by 24 immunoglobulin-like (Ig) repeats. Two flexible hinges separate the 24 Ig repeats into rod 1 (repeats 1-15), rod 2 (repeats 16-23), and self-association domain 24. Rod 1 is like a linear array of Ig repeats, whereas rod 2 is more compact due to inter-domain interactions. FLNa not only support the tension of actin network but also interact with many transmembrane and signaling proteins mostly in the rod 2 segment.

Prompted by recent reports suggesting that interaction of FLNa with its binding partners is regulated by mechanical force, we examined mechanical properties of FLNa domains by magnetic tweezers. The three segments of Ig 1-8, Ig 9-15, Ig 16-23 are unfolded at different forces under the same loading rate. Remarkably, we found that repeats 16-23 are susceptible to ~10 pN force, while the repeats in the rod 1 segment can withstand significantly higher forces.

In rod 2, nearest neighboring domains 16-17, 18-19, and 20-21 form domain pairs. Cryptic binding sites in rod 2 can be blocked by inter-domain interactions. For example, A strand of domain 20 blocks the binding site of β-integrin tail on domain 21. If force can unpeel strand A of domain 20 from domain 21, the binding site of β-integrin tail will get exposed, and facilitate binding. This is just one kind of proposed mechanism of force sensor. The specific domain pair interaction between 20 and 21 was studied using magnetic tweezers. Preliminary data shows that the disruption of the domain pair interaction between domain 20 and 21 occurs at ~15 pN.
Calcium Modulates Force Sensing by the von Willebrand Factor A2 Domain

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Von Willebrand factor (VWF) multimers mediate primary adhesion and aggregation of platelets. The adhesive potency of VWF critically depends on multimer size, which is regulated by a feedback mechanism involving shear-induced unfolding of the VWF-A2 domain and cleavage by the metalloprotease ADAMTS-13. Here we report crystallographic and single-molecule optical tweezers data on VWF-A2 providing mechanistic insight into calcium-mediated stabilization of the native conformation that protects A2 from cleavage by ADAMTS-13. Unfolding of A2 requires higher forces when calcium is present and primarily proceeds through a mechanically stable intermediate with non-native calcium coordination. Calcium further accelerates refolding markedly, and permits active refolding under applied load. Our data support a model in which calcium improves force sensing of VWF by allowing reversible force switching under physiologically relevant hydrodynamic conditions and highlight the relevance of metal coordination for mechanical properties of a protein involved in mechanosensing.
Single Molecule Mechanochemistry for Molecular Insight of Vasculature

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Hemostasis in the arteriolar circulation mediated by von Willebrand factor (VWF) binding to platelets is an example of an adhesive interaction that must withstand strong hydrodynamic forces acting on cells. VWF is a concatenated, multifunctional protein that has binding sites for platelets as well as subendothelial collagen. Binding of the A1 domain in VWF to the glycoprotein Ib a subunit (GPIba) on the surface of platelets mediates crosslinking of platelets to one another and the formation of a platelet plug for arterioles. The importance of VWF is illustrated by its mutation in von Willebrand disease, a bleeding diathesis. Here, we describe a novel mechanochemical specialization of the A1-GPIba bond for force-resistance. We have developed a method that enables, for the first time, repeated measurements of the binding and unbinding of a receptor and ligand in a single molecule (ReLiSM). We demonstrate two states of the receptor-ligand bond, i.e. a flex-bond. One state is seen at low force; a second state begins to engage at 10 pN with a ~20 fold longer lifetime and greater force resistance. The results have important implications not only for how platelets bound to VWF are able to resist force to plug arterioles, but also how increased flow activates platelet plug formation.
Near-Membrane Ensemble Elongation in the Proline-Rich Lrp6 Intracellular Domain may explain the Mysterious Initiation of the Wnt Signaling Pathway

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LRP6 is a membrane protein crucial in the initiation of canonical Wnt/β-catenin signaling pathway, and is dependent on its proline-serine rich intracellular domain for function. LRP6 has five PPP(S/T)P motifs which are phosphorylated during activation starting with the site closest to the membrane. Like all long proline rich regions, there is no stable 3D structure obtained from this isolated and contiguous region. In our study, we use a computational simulation tool to sample the conformational space of the LRP6 intracellular domain under the spatial constraints imposed by (a) the membrane and (b) the close approach of the neighboring intracellular molecular complex assembled on Frizzled when Wnt binds to both LRP6 and Frizzled on the opposite side of the membrane. We observe that an elongated form dominates in the LRP6 intracellular domain structure ensemble. This elongation could relieve conformational auto inhibition of the PPP(S/T)PX(S/T) motif binding sites and allow GSK3 and CK1 to approach their phosphorylation sites, thereby activating LRP6 and the downstream pathway. We propose a model in which the conformation of the LRP6 intracellular domain gets elongated before activation based on the intrusion of the Frizzled complex into the ensemble space of the proline rich region, which alters the shape of its available ensemble space. To test whether this observed ensemble conformational change is sequence dependent, we did a control simulation with a hypothetical sequence with 50% proline and 50% serine in alternating residues. We confirm that this ensemble neighborhood based conformational change is independent of sequence, and conclude that it is likely found in all proline-rich sequences. These observations help us understand the nature of proline rich regions which are both unstructured and which seem to evolve at a higher rate of mutation, while maintaining sequence composition.
Biophysical Aspects of the Interaction of Anticancer and Antibiotic Drugs to Serum Albumin

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The effectiveness of a drug as a pharmaceutical agent depends upon its binding capacity to serum albumins since they are chiefly responsible for delivering the drugs to their target organs. Serum albumins are the most important transport proteins in our body and investigation of the mechanism by which small molecules (drugs in particular) bind, is indispensable for understanding their functioning, drug development and mode of action.

Binding of the anticancer drugs 5-fluorouracil and cyclophosphamide and the antibiotic drug carbenicillin to bovine serum albumin (BSA) has been studied using isothermal titration calorimetry in combination with fluorescence and circular dichroism spectroscopy. The thermodynamic parameters of binding have been evaluated as a function of temperature, ionic strength and in the presence of additives such as anionic, cationic and non-ionic surfactants, tetrabutylammonium bromide and sucrose. Binding affinity of carbenicillin with BSA is observed to be of the order of $10^3 \text{M}^{-1}$ while that of 5-fluorouracil and cyclophosphamide is of the order of $10^2 \text{M}^{-1}$, with the binding profiles fitting well to the single set of binding site model. The values of van’t Hoff and calorimetric enthalpies have been compared to assess the two-state nature of binding. The thermodynamic parameters accompanying the binding of these drugs in presence of additives suggest predominant involvement of electrostatic and hydrophobic interactions in complexation. A support to calorimetric results has been obtained from steady state and lifetime fluorescence measurements along with circular dichroism spectroscopic measurements.
Investigation of POPX2 Phosphatase Functions by Comparative Phosphoproteomic Analysis

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Phosphorylation of proteins is a major post translational modification which regulates many cellular processes, including gene expression, disease mechanisms and signalling pathways. Identification of substrates or biochemical pathways regulated by a phosphatase has always been challenging. Here, we report the use of phosphoproteomic methods to analyse the pathways regulated by POPX2 phosphatase which is a negative regulator of PAK kinase. POPX2 is a serine/threonine phosphatase found in many cancer types. The levels of POPX2 have been found to be up-regulated in the more invasive breast cancer cells compared with non-invasive ones. Our observations also suggest that POPX2 level is positively correlated to cell motility and invasiveness. Thus, finding substrates or pathways regulated by POPX2 will help to elucidate the regulatory mechanism of cancer cell motility and invasiveness. In this study, we have also developed and validated a protocol using electrostatic repulsion-hydrophilic interaction chromatography (ERLIC) to enrich the phosphopeptides followed by LC-MS/MS to allow comparison between the phosphoproteomes of control and POPX2 overexpressing cells. Over 900 non-redundant phosphopeptides were characterized, and phosphoproteins in response to POPX2 overexpression were highlighted. Using the current approach of uncovering the phosphoproteome, we are able to identify biochemical pathways which POPX2 impinges on to exert its cellular function.
A Single Molecule Study of Formin Mediated Actin Polymerization Under Mechanical Forces

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Formin is a family of actin barbed end binding proteins that promote actin nucleation and polymerization. Their association allows continuous polymerization and processive capping of the barbed end. Theoretically, it can be predicted that a stretching force applied on formin may significantly enhance actin polymerization by reducing the critical concentration and increasing the polymerization rate. To experimentally study the effects of mechanical forces on formin mediated actin polymerization, we developed two force application methods that are based on magnetic tweezers and laminar flow respectively. These approaches enable our manipulation of actin filaments and the further study of force dependent actin dynamics in single molecule level.
Regulation of integrin affinity on cell surfaces

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Lymphocyte activation triggers adhesiveness of lymphocyte function-associated antigen-1 (LFA-1; integrin αLβ2) for intercellular adhesion molecules (ICAMs) on endothelia or antigen-presenting cells. Whether the activation signal, after transmission through multiple domains to the ligand-binding αI domain, results in affinity changes for ligand has been hotly debated. Here, we present the first comprehensive measurements of LFA-1 affinities on T lymphocytes for ICAM-1 under a broad array of activating conditions. Only a modest increase in affinity for soluble ligand was detected after activation by chemokine or T-cell receptor ligation, conditions that primed LFA-1 and robustly induced lymphocyte adhesion to ICAM-1 substrates. By stabilizing well-defined LFA-1 conformations by Fab, we demonstrate the absolute requirement of the open LFA-1 headpiece for adhesiveness and high affinity. Interaction of primed LFA-1 with immobilized but not soluble ICAM-1 triggers energy-dependent affinity maturation of LFA-1 to an adhesive, high affinity state. Our results lend support to the traction or translational motion dependence of integrin activation.
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